Global Warming in Freshwaters: Implications for the Microbial-Meiofaunal Loop

Rebecca Stewart

School of Biological and Chemical Sciences Queen Mary,

University of London

Submitted for the degree of Doctor of Philosophy of the University of London September 2012

Abstract

Climate change can have potentially catastrophic effects upon biodiversity and food web structure and according to the fourth IPCC report, ambient temperatures will rise by between 3.0 -5.0 °C over the next century, with already an average increase in global surface temperature of ~0.74°C in the past 100 years. This has known implications in ecology from individuals to ecosystems. The microbial loop consists of small organisms ranging in body size from bacteria (1-15 μm), single-celled eukaryotes (10-1000 μm) and multicellular organisms (250 – 1000 μm) that assimilate dissolved organic carbon into the "classical food web".

The principal goal of this thesis was to assess how rising global temperatures might impact the natural microbial assemblages in 20 mesocosms under 2 treatments – 10 warmed (in line with IPCC predictions) and 10 ambient. The abundance and body mass of 4 major microbial loop taxa (desmids, flagellates, heterotrophic protists and meiofauna) were quantified at monthly intervals over a 2-year period. Secondly, in a microcosm experiment, the population dynamics of three pure cultures of ciliates were monitored across a temperature gradient; the rate of population decline under starvation and changes in body size were quantified.

Results showed that (1) rising global temperatures alters the size spectrum in the autotrophic protists, (2) temperature interacts with temporal and spatial gradients, resulting in changes in phenology (3) these changes in phenology are observable at both the community level and the population level

within the microbial assemblage of the mesocosms and (4) extinction rates and body mass reduction in experimental microcosms were faster at warmer temperatures and partially support predictions of the metabolic theory of ecology.

The implications of these findings are discussed in terms of (1) continued research into the role that small organisms play in community and ecosystem ecologyand (2) the use of these small organisms in experiments as models to inform ecological theory by scaling up from microcosms and finally, (3) I discuss future directions in freshwater microbial ecology, focusing on the increased use of molecular techniques.

Acknowledgements

I am most indebted to my supervisor, Dr. Guy Woodward, for his help, advice and invaluable input in the whole project, discussion and not least, good company throughout my PhD. I am also very grateful to my panel members, Dr Mark Trimmer and Dr. Jonathon Grey for their advice and constructive criticism over the last 4 years. I also thank Dr. Julia Reiss, Dr. Genoveva Esteban and Prof. Bland Finlay for introducing me to the fascinating world of protists and meiofauna in the first place and the use of their laboratory and microscopes over the time I spent at the FBA River Lab in Dorset. Whilst there, Dr. Iwan Jones, Dr. Rasmus Lauridsen, Dr. James Pretty, Adrianna Hawczek and Charles Duerdoth provided me with practical help and valuable friendship that I am thankful for.

Chapter 6 of this thesis was made possible by Dr. Owen Petchey and I would like to thank him for his calm and careful direction in the lab and when using R and for hospitality during the time I spent at Sheffield University.

I am especially grateful to Doris Pichler, Michelle Jackson, Simon Renny-Byfield, Phil Sanders, Gareth Jenkins and Rick Hayes for help, advice aswell as excellent company and lasting friendship over my 4 years at Queen Mary. It would not have been as enjoyable without them all to share it with.

Finally, I am eternally grateful to my extremely tolerant family; my parents, Susan and Dave and my brother, Rob, for their unconditional love, support, irrefutable belief in me and for giving me the opportunity to study in the first place.

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General Introduction

1.1 Climate change

The Earth has experienced many periods of significant climate change over millions of years [(e.g. cooling during the Eocene period (Zachos *et al.* 2001; Miller *et al.* 2005)] The atmosphere has remained relatively stable for the past ~11,500 years during, what geologists refer to as, the Holocene period in which human civilisation began to develop and thrive, so much so that the planet is now entering a new era, termed the Anthropocene (Crutzen 2002). Throughout this period, human activity has had a progressively negative impact on the planet at multiple levels of organisation, which has resulted in rising global temperatures, habitat destruction and fragmentation, ocean acidification, disruption to biogeochemical cycles and the loss of biodiversity (Rockström *et al.* 2009a,b; Steffen *et al.* 2007). One of the most important challenges in ecology is to understand and predict the likely consequences of anthropogenic climate change, yet we are still surprisingly poorly equipped to do so (Walther 2010). This is partly because climate change operates at large spatiotemporal scales and is also likely to interact with other anthropogenic stressors that are already imposed across the planet (Woodward *et al.* 2010; O'Gorman *et al.,* 2012)

Climate change research to date has addressed the effects of anthropogenic stressors at lower levels of organisation (e.g. individuals and populations), but recently, the focus of ecological studies has shifted towards higher, multi-species levels [e.g. communities, food webs, ecosystems (Walther

2010; Woodward *et al.* 2010; O'Gorman et al. 2012)]. The shift in focus is because of the increasing recognition that responses of these systems, in the face of climate change, is more than an aggregation of the effects on individuals (Melian *et al.* 2011). In fact, these systems are comprised of individuals whose ecological and biological characteristics (e.g. life history, body size) are easily measured but to make accurate predictions, it is essential to study how these individuals interact under environmental change so that ecologists can better understand higher-level phenomena. It is therefore imperative that we further our understanding of how environmental changes (e.g. warming) affect individuals, populations, communities and ultimately, entire ecosystems. This is primarily because structural changes may alter key ecosystem processes (e.g. primary production, decomposition, toxin removal) that underpin the valuable ecosystem goods and services (i.e. food, fuel, drinking water) that human society depends on. An important focus for modern ecology is also to ascertain to what extent already-damaged ecosystems are able to maintain essential functions in the long-term and to make predictions regarding the future of these systems and the services they provide.

The fourth IPCC report states that global surface temperatures have increased by ~0.74°C in the past 100 years and predict, under global change scenario A1FI, for temperate latitudes, that ambient temperatures will rise by a further 3-5 °C (average ~4 °C) over the next century (Houghton 2001, 2005; IPCC 2007). Temperature increase is the most familiar and biologically profound change because all biological rates are temperature dependent, from biochemical reactions at the molecular level (Brown *et al.* 2004) to whole ecosystem respiration (e.g. Yvon-Durocher *et al.* 2010a, Yvon-Durocher *et al.*

2012). In addition, temperature sets the pace of life by determining the metabolic rate of individual organisms (Brown *et al.* 2004), with ramifications for higher levels of organisation (Moya-Laraño *et al.* 2012). Potential effects on organisms include changes in (1) phenology and physiology; (2) range and distribution of species; (3) composition of and interactions within communities and (4) the structure and dynamics of communities (Walther *et al.* 2002; Parmesan and Yohe 2003; Parmesan 2006). More climate change studies have been carried out in terrestrial systems than either marine or freshwater systems (Parmesan 2006) and much research is focused on the phenology and physiology of individuals and species range shifts.

Parmesan and Yohe (2003) estimated that 59% of 1598 species in terrestrial systemsdisplayed measurable changes in phenology and/or distribution with a direct link to seasons. For example, in agriculture, historical records are of planting and harvesting and related/important climatic events (e.g. frosts) dating back hundreds of years (Menzel and Dose 2005). Advancement of spring events has been documented on all but one continent and the result of such changes in phenological response to climate may be that of an asynchrony between interacting species in predator-prey relationships and insect-plant interactions. Furthermore, shifts in abundances and ranges of parasites are beginning to influence humans in terms of disease dynamics and impacts on agriculture (Parmesan 2006).

Severe range contractions, as a result of warming, have been observed for polar and mountaintop terrestrial species as their ranges are already restricted and in some cases, this has resulted in extinction. For example, cloud-forest amphibians have declined or gone extinct on a mountaintop in

Costa Rica (Pounds *et al.* 1999), the Apollo butterfly, *Parnassius apollo* has been declared extinct in France (Desmicion *et al.* 2006) and high numbers of population extinctions in pikas, which inhabit mountaintops in the western United States have been recorded (Beever *et al.* 2003).

From marine communities, ecological and physiological research indicates climate as a key factor in influencing community structure and dynamics (Danovaro *et al.* 2004). Elevated sea temperatures as small as 1 ºC above average have led to coral bleaching and El Niño events (extreme increase in temperature) have increased in frequency and a particularly severe event in 1997-1998 caused bleaching in every ocean, with up to 95% of coral bleaching occurring in the Indian Ocean. The result was the loss of 16% of global coral (Hoegh-Guldberg 1999, 2002). In addition to coral bleaching, range shifts have been observed in copepod communities (Beaugrand *et al.* 2002) and in fish and marine invertebrate communities (e.g. Southward *et al.* 2005), both of which have been attributed to warming of the marine environment. It is still unclear how other meiofaunal groups are impacted, and how the protistan taxa may be indicated and how any of these effects and responses may be linked at population, community, food web and ecosystem level.

This pattern is not confined to marine environments; in freshwater systems, shifts in seasonal patterns have been observed in ectotherms; Booth *et al.* (2011) found evidence of range shifts in fishes and Winder and Schlindler (2004) document shifts in the phytoplankton community as well as a resultant asynchrony between the phytoplankton and zooplankton assemblages in a lake ecosystem. Both studies attribute these shifts to anthropogenic climate change. In terms of the microbial loop specifically, the asynchrony or mismatch in

species such as the diatom *Astrionella formosa* and *Daphnia pulicasa*, resulting in an asynchronous predator-prey interaction (Winder and Schlindler 2004), at the level of the microbial loop. Such mismatching may have critical consequences for all ecosystems, especially if keystone species are affected. In pelagic ecosystems, algae–zooplankton interactions form the basis for energy flux to higher trophic levels (Platt *et al.* 2003).

In addition to the potential effects of phenological changes at the ecosystem level, freshwater systems are thought to be particularly sensitive to climate change being effectively "islands in a terrestrial sea" (Arnell and Reynard 1996) meaning that they are especially vulnerable because (1) they are naturally fragmented and inhabitants have a limited ability to disperse in response to environment changes (2) water and temperature availability are climate dependent and (3) they are already exposed to several environmental stressors (e.g. acidification) and rising global temperatures may exacerbate effects of this. For these reasons, freshwater systems are useful and informative study systems from which to infer the effects of climate change in general (e.g. Woodward *et al.* 2010a,b). Current research in a sub-arctic stream system, 30km east of Reykjavik, Iceland (Friberg *et al.* 2009; Woodward *et al.* 2010a,b; Gudmundsdottir *et al.* 2011; O'Gorman *et al.*, 2012) has advanced our understanding of future impacts of warming on freshwater ecosystems in terms of community structure, function and feeding interactions and results from this system, are among the first to address impacts of global warming at higher levels of organisation in fresh waters and using a natural. There are relatively few natural freshwater data sets examining community persistence and there is a gap in our knowledge of the effect at the community and ecosystem level (but

see Woodward *et al.* 2002). However, several mesocosm experiments have addressed impacts of warming at the community or food web level in manipulative experiments (e.g. community: Moss *et al.* 2003, Baulch *et al.* 2005; Yvon-Durocher *et al.* 2010 a,b,c; Dossena *et al.* 2012) and using microcosms (e.g. food webs: Petchey *et al.* 1999; McKee *et al.* 2003; Beveridge *et al.* 2010).

Many studies carried out in natural systems are hampered by confounding latitudinal (or altitudinal) gradients (e.g. Jacobsen *et al.* 1997), such that it may be impossible to disentangle effects of biogeography and temperature. In an attempt to remedy this, investigations into the impacts of warming at the community, food web and ecosystem level in freshwaters have been carried out using laboratory microcosms and experimental mesocosms (e.g. Petchey *et al.* 1999, Yvon-Durocher *et al.* 2010a,b, 2011) and whilst these systems lack the realism and complexity associated with natural systems, they allow high replication, a high level of control and are valuable in assessing effects of warming at higher levels of organisation (see Table 1.2). Despite the abundance of literature and mounting evidence for the far reaching effects of climate change in terrestrial, marine and freshwater systems, gaps remain in our understanding of the effect of climate change on communities and addressing this is of utmost importance in predicting future effects at higher levels of organisation. There are fewer studies of communities and food web ecology still, which include the microscopic organisms that are the focus of this thesis (Robertson *et al.* 2000; Swan and Palmer 2000). In the next section, I address how the microbial loop fits into food web ecology.

1.2 Food webs and the meiofaunal-microbial loop

Ecologists have long sought simple rules to explain the complexity of interactions between individuals, populations and communities. By seeking patterns and generalities in food webs, described by Pimm *et al.* (1991) as the road maps through Darwin's entangled bank, ecologists have been able to illustrate "who eats whom" in natural systems and represent energy flow through from basal levels (primary producers), to primary, secondary and tertiary consumers. As a result, food webs have become an increasingly important level of organisation at which to study how natural systems function (Pascual and Dunne 2005) and how they respond to environmental change (Memmott *et al.* 2005; Dobson *et al.* 2009; Woodward 2009; Woodward et al. 2010a,b). In terms of food webs, the "microbial loop" is an important part of this the diverse complexity in natural systems and comprises small organisms, less than 1mm in size and includes both single-celled organisms [the protozoa (including flagellates, ciliates and amoebae)] and microscopic metazoans (meiofauna). Much research in food web ecology has not included the microbial loop (but see Schmid 2002; Schmid-Araya 2002; Stead *et al.* 2003, 2005; Reiss and Schmid-Araya 2008) and seemingly, the exclusion of these functionally and taxonomically diverse organisms from such studies lacks realism and limits predictions of the consequences of climate change and global warming for whole systems.

The term " microbial loop" was coined by Azam *et al.* (1983) and refers to the continuous cycling of the processes (e.g. carbon sequestration) that these small organisms are thought to be the key drivers of [see (Williams 1981; Azam *et al.* 1983; Hakenkamp and Morin 2000). Research into microbial loop

organisms was initiated by the publication of Pomeroy (1974) who suggested that these organisms assimilate dissolved organic carbon (DOC) and make it available to particle feeders at higher trophic levels [i.e. the classic food chain (Figure 1.1)].

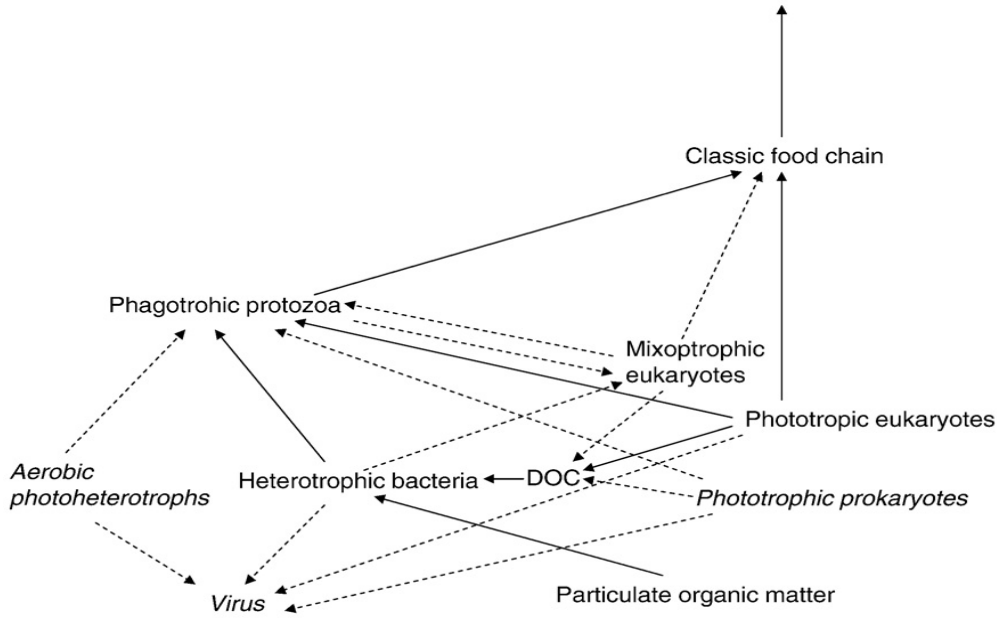


Figure 1.1 The microbial loop as first described by Azam *et al.* 1983, taken from Fenchel (2008). Arrows represent energy and material transfer between functional groups of organisms. And the assimilation of dissolved organic carbon (DOC) into the "classic" food chain.

During the 1970s and 1980s, there was a call for further research into the relevance and importance of the interactions between meiofauna and microbes (Fenchel 1967; Gerlach 1971; Fenchel 1978) and it is now widely accepted that microbes play an important role in ecological systems, being actively involved in essential ecosystem processes, for example, by acting as food sources (Alheit and Scheibel 1982), taking part in mineralisation of nutrients, carbon assimilation (Rafaelli and Mason 1981; Finlay and Esteban 1998; Pomeroy et al. 2007). Important microbial groups include bacteria and microscopic fungi, but as these groups have been considered in detail in recent ecological reviews

(e.g. Ptacnik *et al.*, 2010; Purdy *et al.*, 2010; Reiss *et al.* 2010), I have limited my focus to the protozoa and permanent meiofauna.

Meiofauna are typically defined as either benthic metazoans that range in size from 38-1000*μ*m (Warwick 1984), or those organisms that will pass through a 500 *μ*m sieve but are retained by a 42*μ*m sieve (Robertson *et al.* 2000). Both definitions include "permanent" meiofauna, such as rotifers, tardigrades and gastrotrichs, and also "temporary" meiofauna, such as larval crustaceans and some early- instar insect larvae (Coull and Bell 1979). Rotifers nematodes, meiofaunal-sized testate amoebae and chironomids (temporary meiofauna) tend to be the most species rich groups, according to studies carried out in lotic systems (Robertson *et al.* 2000a,b; Reiss and Schmid-Araya 2008). The ecology and biology of other groups of meiofauna have been documented to some extent in lotic systems (e.g. rotifers: Ricci and Balsamo 2000; microcrustaceans: Dole-Olivier *et al.* 2000; tardigrades: Nelson and Marley 2000; water mites: Sabatino *et al.* 2000) yet some are still relatively poorly understood (e.g. microturbellarians), as highlighted by Robertson *et al.* (2000a).

The Protozoa are a taxon first proposed by Georg August Goldfuss in 1818 and classification has proven difficult until relatively recently (Pomeroy et al. 2007; Fenchel 2008), resulting in the adoption of a broader term, "protista" which includes all protozoa, microscopic algae (desmids and diatoms). According to Corliss (1994), 16 out of 34 protist phyla are thought to live in freshwater, with the following phyla being especially well-represented: Ciliophora (ciliates), Phaeophyta (chrysomonads), Choanozoa (choanoflagellates), Rhizopoda (naked and testate amoebae) and Heliozoa (heliozoans). Finlay and Esteban (1998) provide a comprehensive summary

table of freshwater protist phyla and describe their ecological role in terms of the main food source by species. Within the protozoa, there is a group of organisms, spanning many taxa (including ciliates and amoebae), which share the character of phagotrophy (Fenchel 1986). They are efficient at gathering microbes as food and are small enough to have similar generation times to the food particles on which they feed. These organisms are thought to be the most important grazers of microbes in all aquatic environments and may be the dominant drivers in the control of bacterial abundance (Fenchel 1986; Berninger 1991; Hobbie 1988; Sherr and Sherr 1994; Pomeroy *et al.* 2007) and this makes them a particularly important group to study in natural systems as they provide the link from bacteria to higher taxa, in the classical food web (Figure 1.1). These studies also highlight the taxonomic and functional diversity of this important but as yet, understudied group of organisms, in the context of community and ecosystem ecology, linking food web ecology to these small but important organisms. The study of small organisms has been hindered largely by logistical problems encountered when attempting to carry out accurate enumeration and identification of these organisms (Reiss *et al.* 2010). It is possible that a greater understanding of the small organisms in relation to environmental change will help to discern mechanisms behind recently observed ecosystem level responses to warming (e.g Yvon-Durocher *et al.* 2010 a,b, 2011; Dossena *et al.* 2012) and broaden our understanding of their roles in food webs in general. Given their key role in many ecosystem processes (Hakenkamp *et al.* 2002), it remains important that this role the microbial loop organisms play in natural systems is no longer ignored in community and ecosystem studies, [Figure 1.2 (Schimd-Araya 2002), in

particular, when we compare relative levels of productivity and contribution to biomass (e.g. Nakano *et al.* 1998; Stead *et al.* 2003, 2005; Reiss and Schmid-Araya 2008) but also in making accurate predictions about how whole systems respond under various warming scenarios.

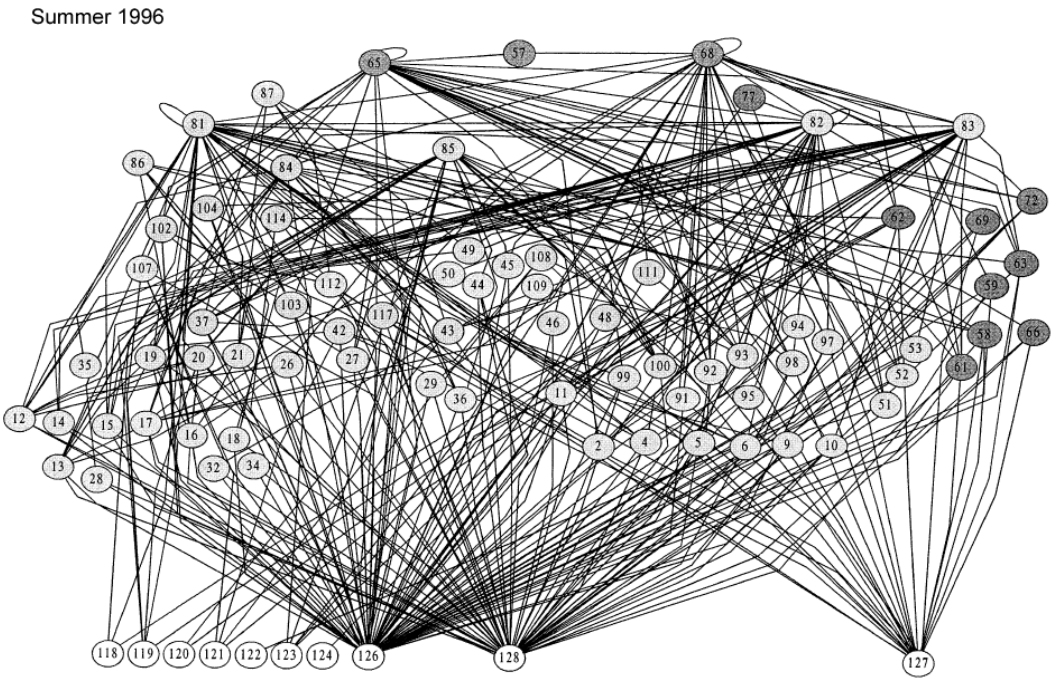


Figure 1.2 Food web from Schmid Araya *et al.* (2002), a study at Broadstone stream, UK that was one of the first studies, in natural systems, to include meiofauna (light grey circles) as well as macrofauna.

Previous studies of climate change in natural systems that have focused on microbial loop organisms have found strong responses of the meiofauna, for example, Price and Warwick (1980) describe marked differences in the respiration rates of a harpacticoid copepod and a sabellid polychaete with increased temperature. Despite the difference between species, the rate increases were approximately represented by a straight line, indicating a

general pattern across species as a direct result of warming. DeNicola (1996) reported clear taxonomic shifts within marine microbial assemblages with increased temperature and nutrient treatments, suggesting interactive effects of temperature with other factors. Other studies have also found clear annual patterns of abundance and species succession (Gasol et al. 1991; Schmid-Araya 1994,1997; Yozzo and Smith 1995; Coull 1999; Aberle et al. 2007). Being intimately associated with the sediment throughout their life cycles, protists and meiofauna provide an effective means of characterising effects of environmental change (e.g. Fenchel and Finlay 1995; Bongers and Ferris 1999). The short life histories of meiofauna faciliates the investiagation of responses to environmental change, over several generations of organisms, within relatively short periods of time (Coull 1999) and thus makes them excellent candidates for testing ecological theory in a controlled laboratory setting.

Whilst understudied due to logistical constraints, in natural systems, small organisms have been widely used in microcosm and mesocosm experiments in the laboratory setting; they have been proved to be useful and productive study organisms when addressing general ecological theory, over the last 15 years (e.g. Lawton 1995; Petchey *et al.* 1999; Petchey *et al.* 2002; Delaney *et al.* 2003; Jessup *et al.* 2004; Bonsall and Hassell 2005; Cadotte *et al.* 2005; Yoshida 2005; Newsham and Garstecki 2007; Pascoal *et al.* 2010; for a thorough, competent review: Reiss *et al.* 2010 and references therein). The value of these studies has been the subject of some debate with advocates (e.g. Benton *et al.* 2007) emphasising the high degree of replication and control that is a notable advantage of an experimental microcosm approach and skeptics of such methods (e.g. Carpenter *et al.* 1996) highlighting that such

studies lack realism when attempting to use these results to inform ecological theory.

Baulch *et al.* (2005) used a mesocosm experiment to determine that higher temperatures resulted in increased bacterial biomass and reduced the quality of food available to higher trophic levels, indicating that warming does have an important effect on the biomass of basal species with effects that will ramify to higher levels of organisation. In a microcosm study, Beveridge *et al.* (2010) investigated direct and indirect effects of warming on predator-prey dynamics in protist microcosms and results they obtained highlight the importance of direct and indirect effects of temperature, mediated through trophic interactions and physical changes in the environment, both for population dynamics and ecosystem processes. Several mesocosm studies have shown an imbalance in the responses of photosynthesis and respiration to warming in mesocosm experiments (Hockelman and Pusch 2000; O'Connor *et al.* 2009; Yvon-Durocher *et al.* 2010b). Pomeroy and Wiebe 2001 show, that in algae and bacteria, respiration rate increase exponentially with warming and that this resulted in increased respiration and decreased photosynthesis (indicated by a measurable switch from autotrophy to heterotrophy). This switch towards greater heterotrophy is of particular interest as it could, in theory, lead to the depletion of dissolved organic carbon within a system (especially closed systems) and impact upon the wider food web (e.g. Baulch *et al.* 2005), the community and potentially the structure of the entire ecosystem, with visible effects on the balance of respiration and photosynthesis (Yvon-Durocher *et al.* 2010a). In this study, I aimed to measure the relative abundance and biomass of autotrophic and heterotrophic microbial organisms, which may reflect this

shift towards greater heterotrophy within the microbial community, as result of warming, whether directly, via effects on microbial communities and populations, or indirectly, via interations with other factors such as seasonal and spatial elements.

1.3 The role of body size, temperature and metabolism

In recent years, reduced body size in the face of rising global temperatures has been coined as the third ecological response to climate change alongside changes in phenology and species range shifts (Angilletta and Dunham 2004; Daufesne *et al.* 2009; Sheridan and Bickford 2011) and there is a commonly observed decline in body size across a wide range of ectothermic taxa (Walters and Hassall 2006).

Elton (1927) wrote, “size has a remarkably great influence on the organisation of animal communities” (p. 59, Elton 1927). He described a ‘pyramid of numbers’ (p. 68) where the numerical abundance of organisms is determined by body size because, “the enemy is larger than the animal upon which it preys” (p. 62). Body size of organisms ranges from the smallest bacteria (10^{-12} g) to the largest whale (10^{8} g) and characteristics such as growth rate, population density and life span change consistently with body size, in allometric scaling relationships (Peters 1983). This also influences the occurrence and consequences of the ecological interactions an organism takes part in (Memmott *et al.* 2000). Energy and material flow in natural systems is therefore linked to the biological processes of individuals of the major taxa within the microbial loop as well [Figure 1.3 (Hakenkamp and Morin 2000)] and this in turn, is influenced by temperature (Brown *et al.* 2004). These

relationships have important implications for patterns at the level of populations and communities (Gaston and Lawton 1988, Lawton 1991, Petchey *et al.* 2008) and have been used as a structural mechanism within and across many levels of organisation (Peters 1983, Kerr and Dickie 2001, Brown *et al.* 2004). In this way, body size relationships have been used to describe interactions between individuals (e.g. between predator and prey) and to find patterns and common allometries in the structure of biological communities (communities: e.g. Cohen *et al.* 2003; Brown *et al.* 2004; Savage *et al.* 2004; Jonsson *et al.* 2005; Reuman and Cohen 2005; Reuman *et al.* 2008; Woodward *et al.* 2005a,b; between ecosystem types: Yvon-Durocher *et al.* 2012).

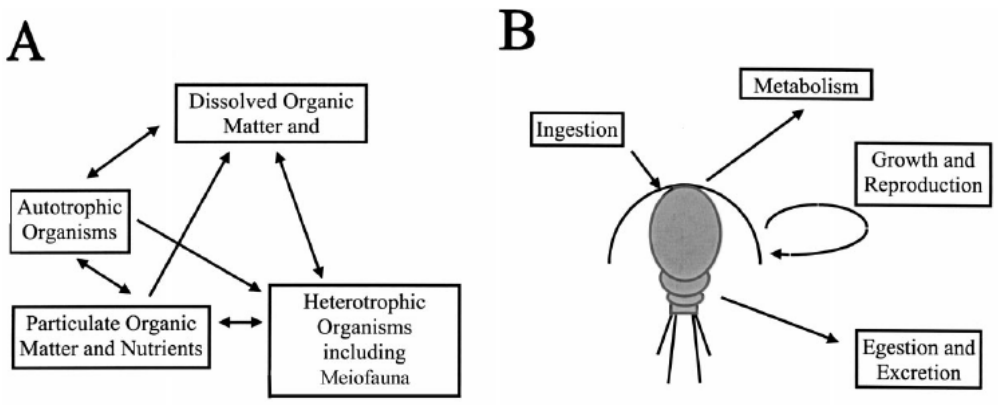


Figure 1.3 From Hakenkamp and Morin (2000). A: Energy and material flow between organisms in streambed sediments. Arrows indicate direction of flow. B: Biological activities if meiofauna that are thought to influence organic matter and nutrient flux. These processes are also affected by temperature (Brown *et al.* 2004).

Allometric scaling patterns link population level patterns to the metabolic needs of the individual and tend to a simple power function: $\mathbf{y = k\ M^b}$, where **y** is the characteristic of interest, **k** is the allometric constant, **M** is body mass and **b** is the allometric scaling component (Thompson 1917; Huxley 1932).

According to Bergman's rule, organisms tend to be larger in colder regions (Bergman 1847, Ray 1960, James 1970, Ashton *et al.* 2000, Ashton 2002), which also suggests that global warming may alter the distribution of body sizes via species range shifts (Chen et al. 2011) and/or physiological adaptation (Musolin 2007). Several explanations, which are not mutually exclusive, have been proposed for warming favouring the small (Daufresne et al. 2009). These include James's Rule, which predicts that the mean body size of a species population will decline with temperature (James 1970) and a subset of James's Rule, the Temperature-Size Rule (TSR) which predicts that oxygen demands and different thermal sensitivities in growth and development rate will lead to smaller size, at later developmental stages, at warmer temperatures (Ray 1960; Atkinson 1994,1995; Berrigan and Charnov 1994; Angilletta and Dunham 2004; Forster *et al.* 2011). The link between these non-mutually exclusive theories is that of body size and metabolism and response to temperature (in the case of this thesis. Where Bergman's Rule is specific to mammals and birds, TSR and MTE may be applied to ectotherms and endotherms (see Table 1.1).

With TSR, the different thermal sensitivity in growth and development are postulated to be because individual growth and development rates are dependent on both body size and temperature (Angilletta and Dunham 2004). It may be that faster growth favours early maturity at small body size if the coefficient of growth and asymptotic size are negatively related, as supported by the differential effects of temperature on anabolism and catabolism (von Bertalanffy 1960, Perrin 1995). Thermal constraints on maximum body size can limit growth late in ontogeny, reducing the benefit of delayed maturation

(Berrigan and Charnov 1994, Kindlmann *et al.* 2001) As a result, greater fecundity associated with larger body size (Stearns 1992, Roff 2002) may be selected for in cold environments (Angilletta and Dunham 2004).

The “Metabolic Theory of Ecology” (MTE), as proposed by Brown *et al.* (2004) attempts to explain the relationship between metabolic rate and body size and temperature of every animal, plant and microbe. The core assumption of the theory is that anatomical and physiological traits are mechanistically linked to variation in branching, fractal-like vascular networks with the size of the organism in question (West *et al.* 1997, 1999). In short, it may provide a useful link between the biochemistry (metabolism) of any individual organism with the ecology of populations, communities and ecosystems. The main prediction of the MTE is that many biological rates follow a thermal response modeled by the Arrhenius function (Brown *et al.* 2004). This sets rates of resource uptake and allocation of resources to growth, survival and reproduction and ultimately influences ecological processes at the level of the individual and that this filters upwards through other more complex levels of organisation, including communities, food webs and ecosystems (Brown *et al.* 2004; Allen *et al*. 2005).

Conversely, Van der Have and de Jong (1996) proposed that differential temperature dependencies in growth and development rates determine size at maturity. Here, if the effect of temperature is greater on development rate than on growth rate, warming should lead to a smaller adult size (Smith 1979, van der Have and de Jong 1996, Davidowitz and Nijhout 2004, Walters and Hassall 2006, Forster*et al.* 2011). This suggests that underlying assumptions of MTE, related to many biological rates following a thermal response modelled by the

Arrhenius function (Brown *et al.* 2004), may not be complete and this could explain these observed exceptions to the Temperature-Size Rule (van der Have and de Jong 1996, Walters and Hassall 2006). Further, recent models of eco-evolutionary food web dynamics suggest that warm environments might not necessarily always favour smaller organisms (Moya-Laraño *et al.* 2012). Table 1.1 provides a summary of the theories described here relating to warming. Small organisms are excellent candidates for testing these theories and this thesis attempts to link these theories with organisms of the microbial loop as part of a long-term mesocosm experiment and a laboratory microcosm experiment.

1.4 Previous work in the mesocosms

Mesocosm experiments represent a compromise between the control and replication of laboratory studies and the realism of descriptive field surveys but, despite their limitations, they can provide a useful tool for predicting how global change scenarios might affect ecosystem level processes [see Table 1.2 (Benton *et al.* 2007)]. The long-term mesocosm experiment used in this experiment (Figure 1.4) has been used to address warming in several previous studies (Yvon-Durocher *et al.* 2010a,b, 2011; Dossena *et al.* 2012) and the set-up is described, in full, in *chapter 2* of this thesis.

Figure 1.4 The global warming mesocosm experiment in February 2009. The experimental plot consisted of 20 mesocosms: 10 heated and 10 unheated

Yvon-Durocher *et al.* (2010 a,b,c) used the same experimental mesocosms as this study to investigate the effect of warming on whole ecosystem respiration and gross primary productivity [(Yvon-Durocher *et al.* 2010a) Figure 1.5] and on the size spectra of phytoplankton and zooplankton assemblages [(Yvon-Durocher *et al.* 2011) Figure 1.6]. A later study by Dossena *et al.* (2012), using the same mesocosms, found significant shifts in the size structure of the benthic community (macroinvertebrates), with increased abundance of small-bodied organisms in the spring and reduced abundance of smaller bodied organisms in the autumn. They also linked this shift in the community size spectrum to the functioning of the ecosystem as the size spectrum shifts were mirrored by shifts in the decomposition rates in the benthos. These studies revealed interesting and profound influence of temperature on the size structure and metabolism of the systems, with results that are relevant to the studies carried out in this thesis. One logical progression from these existing studies is to investigate further, the effect of temperature on the population dynamics and community structure of the small organisms that dominate the mesocosms and are known

to play signigficant roles in ecosystems and to investigate whether the patterns observed by these previous studies are apparent within the protist and meiofauna community and populations. For example, Figure 1.5 shows a shift towards increased heterotrophy in the warmed ponds as the ratio of respiration to photosynthesis increases. The microbial loop organisms may aid explanation of this result if, for example, there is an increase in heterotrophic to autotrophic genera and species within this large group.

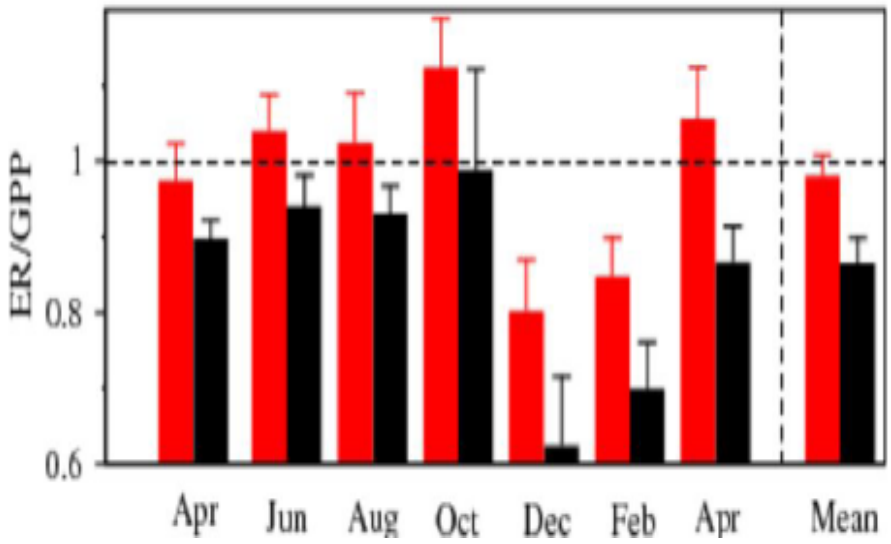


Figure 1.5 From Yvon-Durocher *et al.* (2010a). Warming resulted in a significant shift towards increased heterotrophy in warmed (red bars) ponds compared to ambient ponds (black bars). Shown by an increase in respiration relative to photosynthesis.

Secondly, the shift in size spectra is particularly interesting in light of current theories regarding reduced body size as the third universal response to climate change (Angiletta and Dunnham 2003), as demonstrated by Yvon-Durocher *et al.* (2010c) in the mesocosms. This study attempts a further investigation of this phenomenon will focus on the microbial loop will further our understanding of freshwater systems in a warmer world.

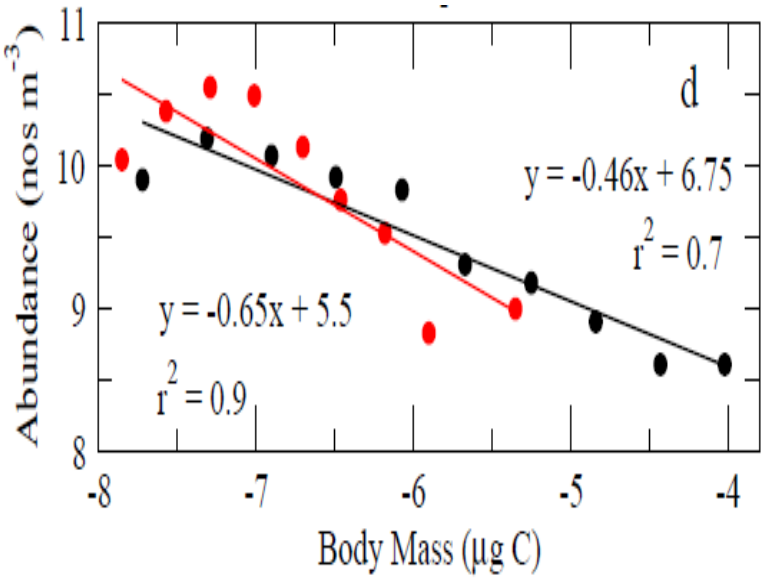


Figure 1.6 From Yvon-Durocher *et al.* (2010c). Warming resulted in a significant shift in the size spectra of phytoplankton, favouring smaller individuals in the warmed ponds (red points) compared to ambient ponds (black points).

Table 1.1 A summary of the theories relating to warming and body mass, describing the level at which they apply and the mechanism by which they operate. In this thesis, I tested James's Rule and the Temperature Size Rule (TSR) as well as the Metabolic Theory of Ecology (MTE) using microscopic organisms.

Rule/hypothesis	Level of operation	Mechanism	Metabolic Type	Scaling exponents	Reference
Bergmann's Rule – Species shift hypothesis	Population, community level	Evolutionary	Endo- and ectotherms	Two-thirds mass scaling	Blackburn *et al.* 1999; Daufesne *et al.* 2009
James' Rule – Population-age structure shift	Population level	Evolutionary, Ecological	Endo- and ectotherms	Two-thirds mass scaling	Daufesne *et al.* 2009
Temperature Size Rule – Size-at-age and Size-at-Stage	Individual, population level	Developmental, Ecological	Ectotherms	Two-thirds mass scaling	Walters and Hassall 2006; Daufesne *et al.* 2009
Metabolic Theory of Ecology	Individual, ecosystem level	Ecological	Endo- and ectotherms	Quarter or three-quarter power mass scaling.	Brown *et al.* 2004; Savage *et al.* 2004

Table 1.2 Advantages and disadvantages of different scales of ecological experiments used to answer global change questions. This thesis focuses on field mesocosms and laboratory microcosms.

Scale	Advantages	Disadvantages
Microcosms	Easy to manipulate environmental variables	Difficult to scale up to natural systems
	High level of replication	Only focus on a subset of species/traits
	Focus on specific mechanisms	Short temporal duration
Field Mesocosms	Intermediate replication level	Closed systems
	Produce realistic community structure	Short temporal duration
	Focus on specific mechanisms	
Field Manipulations	Natural community composition	Low level of replication
	Reduced realism issues	Perturbation may be unrealistic
		Confounded by co-variables e.g nutrient limitation
Spatial Field Surveys	Natural community composition	Confounded by co-variables e.g. biogeography
	Incorporates natural stochasticity	Long temporal series required

1.5 Goals of this study: aims and hypotheses

The principal aim of this study was to investigate the impact of warming on the abundance and biomass of the microbial community and, to discuss the potential impact responses at the microbial level might have at higher levels of organisation. Using a combination of a freshwater mesocosm experiment (described above) and a laboratory microcosm experiment, I tested current theories (predominantly the TSR and MTE) regarding individual, population and community responses to rising global temperatures and later, and discussed the wider implications and potential mechanisms driving shifts in the microbial loop.

I sampled the microbial community in 20 freshwater mesocosms, 10 of which were under warming treatment (see methodology in *chapter 2*), and analysed community abundance, biomass, community composition and body mass-abundance relationships over a yearlong sampling period (*chapters 3,4 and 5* of this thesis). I collected quantitative data on individuals of the microbial loop sampled from every pond, every month from February 2009 until January 2010.

To test the predictions of the MTE, I carried out a microcosm experiment to investigate the relationship between temperature, mass and rate of population decline in 3 “axenic” (pure) populations of protists.

Chapter 3 of this thesis (“Size spectra and allometries of microbial-meiofaunal assemblages in experimentally warmed mesocosms”) focuses on the size spectra and allometric scaling of microbial-meiofaunal assemblages in the

mesocosms using individual based size spectra of the community, irrespective of taxonomy and I asked the following questions;

1. Does the slope of the community size spectrum (mass-abundance relationship) change with warming, in line with classic size spectrum theory (White *et al.* 2007) and allometric scaling theories [e.g. the MTE (Brown *et al.* 2004)]?

2. Is the size spectrum truncated among autotrophs and heterotrophs, as has been observed in previous studies in the same mesocosm experiment (Yvon-Durocher *et al.* 2010c)?

Chapter 4 describes the broader community composition of the assemblages, using taxonomic-averaged mass and abundance data and attempted to answer the following questions:

1. How does warming affect the abundance and biomass of major microbial-meiofaunal taxa?

2. Does warming favour smaller species within major taxa of the microbial-meiofaunal community in the mesocosms, as predicted by the MTE (Brown *et al.* 2004) and the TSR (Atkinson 1994; Daufresne *et al.* 2009)?

In Chapter 5, I investigated population level shifts in size and abundance in response to warming in the mesocosms, focusing on dominant species and testing warming theories such as the TSR and address 2 principal questions:

1. How does warming affect the abundance and biomass of populations of microbial and meiofaunal genera in the mesocosms?

2. Are individuals within a species smaller as a result of warming, in support of James's rule (1970).

Chapter 6 contains results from a microcosm experiment to investigate the connections between individual body mass, temperature and rate of population decline using the MTE predictions to explain observed patterns. This chapter addresses the following questions;

1. How does temperature influence population decline rates (to extinction) of pure populations of protists in laboratory microcosms?

2. Can population decline rates be predicted by temperature and mass dependence of biological rates, as predicted by the MTE (Brown *et al.* 2004).

1.6 References

Aberle N, Lengfellner K, Sommer U (2007) Spring bloom succession, grazing impact and herbivore selectivity of ciliate communities in response to winter warming. Oecologia **150**:668–681

Alheit, J. and Scheibel, W. (1982) Benthic harpacticoids as a food source for fish *Marine Biology*, **70,** 141-147

Allen, A.P, Gillooly, J.F and Brown, J.H (2005) Linking the global carbon cycle to individual metabolism *Functional Ecology* **19,** 202-213

Angilletta, M.J., and Dunham, A.E. (2004) The temperature-size rule in ectotherms: Simple evolutionary explanations may not be general*Am. Nat.* **162**, 332–342

Arnell, N.W.and Reynard, N.S. (1996) The effects of climate change due to global warming on river flows in Great Britain*Journal of Hydrology*, **183,** 397-424

Ashton, K.G. (2002) Patterns of within-species body size variation of birds: strong evidence for Bergmann's rule. *Global Ecology and Biogeography* **11**, 505-523

Ashton, K.G., Tracy, M.C., and Queiroz, A.D.(2000) Is Bergmann's Rule valid for mammals? *The American Naturalist* **156**, 390-415

Atkinson, D. (1994) Temperature and organism size—A biological law for ectotherms *Adv. Ecol. Res.* **25**, 158

Atkinson, D. (1995) Effects of temperature on the size of aquatic ectotherms: Exceptions to the general rule *Journal of Thermal Biology* **20**, 61-74

Atkinson, D., Ciotti, B. J., and Montagnes, D. J. S. (2003) Protists decrease in size linearly with temperature: ca. 2.5% °C–1. *Proceedings of the Royal Society of London. Series B: Biological Sciences* **270**, 2605-2611

Azam, F., Fenchel, T., Field J.G., Gray, J.S., Meyerreil, L.A.and Thingstad, F. (1983) The ecological role of water column microbes in the sea *Marine Ecology-Progress Series*,**10,** 257-263

Badeck, F.W., Bondeau, A., Bottcher, K., Doktor, D., Lucht, W., *et al.* (2004) Responses of spring phenology to climate change *New Phytol.* **162,** 295-309

Baulch, H.M., Schindler, D.W., Turner, M.A., Findlay, D.L., Paterson, M.J. and Vinebrooke, R.D. (2005) Effects of warming on benthic communities in a boreal lake: Implications of climate change*Limnology and Oceanography*, **50,** 1377-1392

Beever, E.A., Brussard, P.F., Berger, J., (2003) Patterns of apparent extirpation among isolated populations of pikas (*Ochotona princeps*) in the Great Basin. *J. Mammal.* **84**: 37-54

Beaugrand, G., Reid, P.C., Ibanez, F., Lindley, J.A., Edwards, M., (2002) Reorganization of North Atlantic marine copepod biodiversity and climate. *Science* **296**: 1692-94

Benton, T.G., Solan, M., Travis, J.M.J. and Sait, S.M. (2007) Microcosm experiments can inform global ecological problems *Trends in Ecology and Evolution* **22,** 516-521

Bergmann, C. (1847) Uber die Verhaltnisse der Warmeokonomie der Thiere zu ihrer Grosse*Gottinger Studien* **3**, 595–708

Berrigan, D. and Charnov, E. L. (1994) Reaction norms for age and size at maturity in response to temperature: a puzzle for life historians *Oikos* **70**, 474-478

Berninger, U.G., Finlay, B.J. and Kuuppoleinikki, P. (1991) Protozoan control of bacterial abundances in fresh-water *Limnology and Oceanography*, **36,** 139-147

Beveridge, O.S., Humphries, S., and Petchey, O.L. (2010) The interacting effects of temperature and food chain length on trophic abundance and ecosystem function *J. Anim. Ecol.* **79**, 693–700

Blackburn, T.M., Gaston, K.J. and Löder, N. (1999) Geographic gradients in body size: a clarification of Bergmann's rule *Diversity and Distributions,* **5**, 165– 174

Bongers, T. and Ferris, H. (1999) Nematode community structure as a bioindicator in environmental monitoring *Trends in Ecology and Evolution*, **14,** 224-228

Bonsall, M.B., and Hassell, M.P. (2005) Understanding ecological concepts: The role of laboratory systems*Adv. Ecol. Res*. **37**, 1–36

Booth, D.J., Bond, N., Macreadie, P. (2011) Detecting range shifts among Australian fishes in response to climate change*Marine and Freshwater Research*, **62**, 1027–1042

Brown, J.H., Gillooly, J.F., Allen, A.P., Savage, V.M.and West, G.B. (2004) Toward a metabolic theory of Ecology*Ecology* **85,** 1771-1789

Cadotte, M.W., Drake, J.A., and Fukami, T. (2005) Constructing Nature: Laboratory models as necessary tools for investigating complex ecological communities *Adv. Ecol. Res*. **37**, 333–353

Caron, D.A., Worden A.Z., Countway, P.D., Demir, E. and Heidelberg, K.B. (2008) Protists are microbes too: a perspective *ISME J*, **3,** 4-12

Carpenter, S.R. (1996) Microcosm experiments have limited relevance for community and ecosystem ecology *Ecology* **77**, 677–680

Chen, I.-C., Hill, J.K., Ohlemüller, R., Roy, D.B., and Thomas, C.D. (2011) Rapid range shifts of species associated with high levels of climate warming, *Science,* **333**, 1024

Cohen, J.E., Jonsson, T., and Carpenter, S.R. (2003) Ecological community description using the food web, species abundance, and body size *PNAS* **100**, 1781–1786

Corliss, J.O. (1994) An interim utilitarian (user-friendly) hierarchical classification and characterization of the protists *Acta Protozoologica*, **33,** 1-51

Coull, B.C. (1999) Role of meiofauna in estuarine soft-bottom habitats. *Australian Journal of Ecology,* **24,** 327-343

Coull, B.C. and Bell, S.S. (1979) Nitrocrella-aestuarina n-sp and the male of Mesochra-mexicana (Copepoda, Harpacticoida) from South Carolina salt marshes *Transactions of the American Microscopical Society*, **98,** 219-224

Crutzen, P.J. (2002) The Effects of Industrial and Agricultural practices on Atmospheric Chemistry and Climate dduring the Anthropocene *J. Environ. Sci. Health. A.* **37,** 4, 423-

Damuth, J. (1981) Population-density and body size in mammals. *Nature* **290**, 699-700

Damuth, J. (1987) Interspecific allometry of population density in mammals and other animals: The independence of body mass and population energy-use*Biol. J. Linn. Soc.* **31**, 193–246

Daufresne, M., Lengfellner, K., and Sommer, U. (2009) Global warming benefits the small in aquatic ecosystems*Proceedings of the National Academy of Sciences of the United States of America* **106**, 12788-12793

Danovaro, R., Dell'Anno, A. and Pusceddu, M. (2004) Biodiversity response to climate change in a warm deep sea *Ecology Letters* **7,** 821-828

Davidowitz, G. and Nijhout, H.F. (2004) The physiological basis of reaction norms: the interaction among growth rate, the duration of growth and body size. *Integrative and Comparative Biology* **44**, 443-449

Delaney, M.P. (2003) Effects of temperature and turbulence on the predator-prey interactions between a heterotrophic flagellate and a marine bacterium *Microb. Ecol.***45**, 218–225

DeNicola, D.M. (1996) Effects of solar spectral irradiance (visible to UV) on a prairie stream epilithic community *Journal of the North American Benthological Society,* **5:2**, 155 -169

Descimon H, Bachelard P, Boitier E, Pierrat V (2006) Decline and extinction of Parnassius apollo populations in France – continued. *In: Studies on the Ecology*

and Conservation of Butterflies in Europe (EBIE), ed. E Kuhn, R Feldman, J Settele. Bulgaria: PENSOFT

Dobson, A., Allesina, S., Lafferty, K., Pascual, M. (2009) The assembly, collapse and restoration of food webs *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* **364 (1524**): 1803–1806

Dole-Olivier, M.J., Galassy D.M.P., Marmonier, P., Creuzé des Châtelliers, M. (2000) The biology and ecology of lotic microcrustaceans *Freshwater Biology* **44,** 63-91

Dossena, M., Yvon-Durocher, G., Grey, J., Montoya, J.M., Perkins, D.M., Trimmer, M., and Woodward, G. (2012) Warming alters community size structure and ecosystem functioning*Proc. R. Soc. B.* **279**, 3011-3019

Elton, C. (1927) Animal Ecology *Sidgwick and Jackson, London*

Fenchel, T. M. (1967) The ecology of marine microbenthos. I. The quantitative importance of ciliates as compared with metazoans in various types of sediments *Ophelia* **4**, 121–137.

Fenchel, T.M. (1978) Ecology of microbenthos and meiobenthos Annual *Review of Ecology and Systematics*, **9,** 99-121.

Fenchel, T. (1986) The ecology of heterotrophic microflagellates *Advances in Microbial Ecology*, **9,** 57-97

Fenchel, T. (2008) The microbial loop-25 years later*Journal of ExperimentalMarine Biology and Ecology,* **366,** 99-103

Finlay, B.J and Esteban, G.F (1998) Freshwater protozoa: biodiversity and ecological function *Biodiversity and Conservation***7,** 1163-1186

Forster, J., Hirst, A. G., and Woodward, G. (2011) Growth and development rates have different thermal responses *American Naturalist* **178**, 668-678

Friberg, N., Dybkjær, J.B., Olafsson, J.S., Gislason, G.M., Larsen, S.E., Lauridsen, T.L. (2009) Relationships between structure and function in streams of contrasting temperature *Freshwater Biology* **54**, 2051–2068

Gasol, J.M., Guerrero, R., and Pedrosalio, C. (1991) Seasonal variations in sizestructure and prokaryotic dominance in sulphurous Lake Ciso *Limnol. Oceanogr.* **36**, 860–872

Gaston, K.J. and Lawton, J.H. (1988) Patterns in body size, population dynamics and regional distribution of bracken herbivores*American Naturalist***132**, 662-680

Gerlach, S.A, (1971) On the importance of marine meiofauna for benthos communities *Oecologia* **6,** 176-190

Gudmundsdottir, R. Gislason, G.M., Palsson, S., Olafsson, J.S., Anders Schomacker, A., Friberg, N., Woodward, G., Hannesdottir, E.R., Moss, B (2011) Effects of temperature regime on primary producers in Icelandic geothermal streams *Aquatic Botany* **95**, 278–286

Hakenkamp, C.C. and Morin, A. (2000) The importance of meiofauna to lotic ecosystem functioning *Freshwater Biology* **44,** 165-175

Hakenkamp, C.C., Morin, A. and Strayer, D.L. (2002) The functional importance of freshwater meiofauna. In: Freshwater Meiofauna: Biology and Ecology (Eds S.D. Rundle, A.L. Robertson and J.M. Schmid-Araya), pp. 321–335. *Backhuys Publishers, Leiden*

Hobbie, J.E. (1988) A comparison of the ecology of planktonic bacteria in fresh and salt-water *Limnology and Oceanography* **33,** 750-764

Hockelmann, C.and Pusch, M. (2000) The respiration and filter-feeding rates of the snail Viviparus viviparus (Gastropoda) under simulated stream conditions*Archiv Fur Hydrobiologie* **149,** 553-568

Hoegh-Guldberg, O. (1999) Climate change, coral bleaching and the future of the world's coral reefs. *Mar. Freshw. Res.***50**: 839–66

Hoegh-Guldberg, O. and Bairlein, F. (2002) Ecological responses to recent climate change *Nature,* **416,** 389-395

Houghton, J. (2001) The science of global warming *Interdisciplinary Reviews* **26,** 247-257

Houghton, J. (2005) Global Warming *Reports on Progress in Physics* **68,** 1343-1403

IPCC (2007) in Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*Ed. Parry, M.L., Canziani, O.F., Palutikof, J.P., van der Linden, P.J. and Hanson, C.E. (Cambridge University Press, Cambridge) pp. 7-22*

Jacobsen, D., Schultz, R. and Encalada, A. (1997) Structure and diversity of stream invertebrate assemblages: the influence of temperature with altitude and latitude *Freshwater Biology*, **38**: 247–261

James, F. C. (1970) Geographic Size Variation in Birds and Its Relationship to Climate *Ecology* **51**, 365-390

Jessup, C.M., Kassen, R., Forde, S.E., Kerr, B., Buckling, A., Rainey, P.B., and Bohannan, B.J.M. (2004) Big questions, small worlds: Microbial model systems in Ecology *Trends Ecol. Evol.***19**, 189–197

Kerr, S.R. and Dickie, L.M. (2001) The Biomass Spectrum: A Predator Prey Theory of Aquatic Production, *Columbia University Press, New York*

Lawton, J.H. (1991) Species richness, population abundances, and body sizes in insect communities: tropical versus temperate comparisons *In: Price PW, Lewinsohn TM, Fernandes GW, Benson WW, editors. Plant-Animal Interactions, pp. 71-89. John Wiley & Sons, Inc.*

Lawton, J.H. (1995) Ecological Experiments with Model Systems*Science* **269***,* 328-331

McKee, D., Atkinson, D., Collings, S.E.*, et al.* (2003) Response of freshwater microcosm communities to nutrients, fish, and elevated temperature during winter and summer *Limnology and Oceanography* **48**, 707-722

Melian, C. J., Vilas, C., Baldo, F., Gonzalez-Ortegon, E., Drake, P., and Williams, R. J. (2011) Eco-evolutionary dynamics of individual-based food webs Pages 225-268 *inA. R. J. Belgrano, editor. Advances in Ecological Research, Vol **45**: The Role of Body Size in Multispecies Systems*

Memmott, J., Alonso, D., Berlow E., Dobson, A.P., Dunne, J.A., Sole, R.V., Weitz, J. (2005) Habitat loss and food web structure*In: Dunne J., Pascual M., editors. Ecological networks: linking structure to dynamics. Oxford University Press; Oxford, UK*pp. 235–247

Menzel, A., Dose, V. (2005) Analysis of long-term time-series of beginning of flowering by Bayesian function estimation *Meterol. Z.* **14**, 429-34

Miller, G.H., Fogel, M.L., Magee, J.W, Gagan, M.K., Clarke, S.J., Johnson, B.J. (2005) Ecosystem Collapse in PleistoceneAustralia and a Human Rolein Megafaunal Extinction*Science* **309**, 287

Montoya,J.M. and Raffaelli,D. (2010) Climatechange,biotic interactions and ecosystem services *Phil. Trans. R. Soc. B.*, **365**, 2013–2018

Moran, X.A.G., Lopez-Urrutia, A., Calvo-Diaz, A., Li, W.K.W. (2010) Increasing importance of small phytoplankton in a warmer ocean *Global Change Biology,* **16**, 1137-1144

Moss, B., McKee, D., Atkinson, D., Collings, S.E., Eaton, J.W., Gill, A.B., Harvey, I., Hatton, K., Heyes, T., and Wilson, D. (2003) How important is climate? Effects of warming, nutrient addition and fish on phytoplankton in shallow lake microcosms. *J. Appl. Ecol.* **40**, 782–792

Moya-Loraño, J., (2012) O matrices and eco-evolutionary dynamics *Trends. Ecol. Evol.* **27(3),** 139-40; author reply 140. Epub 2011 Dec 22

Nelson, D.R. and Marley, N.J. (2000) The biology and ecology of lotic tardigrada *Freshw. Biol.* **44,** 93-108

Newsham, K.K., and Garstecki, T. (2007) Interactive effects of warming and species loss on model Antarctic microbial food webs. *Funct. Ecol.* **21**, 577–584

O'Connor, M.I., Piehler M.F., Leech, D.M., Anton, A., Bruno, J.F. (2009) Warming and resource availability shift food web structure and metabolism*PLoS Biol* **7(8)**: e1000178. doi:10.1371/journal.pbio.1000178

O'Gorman, E.J., Pichler, D.E., Adams, G., Benstead, J.P., Craig, N., Cross, W.F., Demars, B.O.L., Friberg, N. Gísli Mar Gíslason[8], Rakel Gudmundsdóttir, R., Hawczak, A., Hood, J.M., Hudson, L.N., Liselotte Johansson, L., Johansson, M., Junker, J.R., Laurila, A., Manson, J.R., Mavromati, E., Nelson, D., Ólafsson, J.S., Perkins, D.M., Petchey, O.L., Plebani, M., Reuman, D.C., Rall, B.C., Stewart, R., Thompson, M.S.A. and Woodward, G. (2012) Impacts of warming on the structure and function of aquatic communities: individual- to ecosystem-level responses *An. Ecol. Rev.* **47,** 81-176

Parmesan, C. and Yohe, G.(2003) A globally coherent fingerprint of climate change impacts across natural systems *Nature* **421**, 37-42

Parmesan, C. (2006) Ecological and evolutionary responses to recent climate change*Annual Review of Ecology Evolution and Systematics* **37,** 637-669

Pascoal, C., Càssio, F., Nikolcheva, L., and Bärlocher, F. (2010) Realized fungal diversity increases functional stability of leaf litter decomposition under zinc stress *Microb. Ecol.* **59,** 84–93

Pascual, M.M. and Dunne, J.A. (2005) Ecological networks: linking structure to dynamics *Oxford, UK: Oxford University Press*

Perrin, N. (1995) About Berrigan and Charnov's life-history puzzle *Oikos* **73**, 137-139

Petchey, O., Mcphearson, P., Casey, T.and Morin, P. (1999) Environmental warming alters food-web structure and ecosystem function *Nature* **402,** 69-72

Petchey, O.L., Morin, P.J., Hulot, F.D., Loreau, M., McGrady-Steed, J., and Naeem, S. (2002) Contributions of aquatic model systems to our understanding of biodiversity and ecosystem functioning *In: Biodiversity and Ecosystem Functioning: Synthesis and Perspectives (Ed. by M. Loreau, S. Naeem and P. Inchausti),* pp. 127–138 *Oxford University Press, Oxford*

Petchey, O.L., Long, Z.T., and Morin, P.J. (2007). The consequences of body size in model microbial ecosystems*In: Body Size: The Structure and Function of Ecosystems (Ed. by Hildrew, A.G., Raffaelli, D. and Edmonds-Brown, R.). Cambridge University Press, Cambridge*

Petchey, O. L., Beckerman, A. P., Riede, J. O., and Warren, P. H. (2008) Size, foraging, and food web structure *Proceedings of the National Academy of Sciences of the United States of America* **105**, 4191-4196

Peters, R.H. (1983) The ecological implications of body size Cambridge University Press, Cambridge

Pimm, S.L., Lawton, J.H., Cohen, J.E. (1991) Food Web Patterns and Their Consequences *Nature*, **350**, 669-674

Platt, T., C. Fuentes-Yaco, and K. T. Frank. (2003) Marine ecology: spring algal bloom and larval fish survival. *Nature*, **423,** 398–399

Pomeroy, L.R. (1974) The Ocean's Food Web, a Changing Paradigm *BioScience,* **24:9**, 499-504

Pomeroy, L.R. and Wiebe, W.J. (2001) Temperature and substrates as interactive limiting factors for marine heterotrophic bacteria *Aquatic Microbial Ecology*, **23,** 187-204

Pounds, J.A., Fogden, M.P.L., Campbell, J.P.H. (1999) Biological response to climate change on a tropical mountain *Nature*, **398**: 611-15

Price, R.and Warwick, R.M. (1980) The effect of temperature on the respiration rate of meiofauna *Oecologia (Berlin)* **44,** 145-148

Ptacnik, R., Moorthi, S.D., and Hillebrand, H. (2010) Hutchinson reversed, or why there need to be so many species*Adv. Ecol. Res.***43**, 1–43

Purdy, K.J., Hurd, P.J., Moya-Laraño, J., Trimmer, M., and Woodward, G. (2010). Systems biology for ecology. *Adv. Ecol. Res.***43**, 87–149

Ray, C. (1960) The application of Bergmann's and Allen's rules to the poikilotherms. *Journal of Morphology* **106,** 85–108

Reiss, J.and Schmid-Araya, J.M. (2008) Existing in plenty: abundance, biomass and diversity of ciliates and meiofauna in small streams *Freshwater Biology*, **53,** 652-668

Reiss, J., Forster, J., Cassio, F., Pascoal, C., Stewart, R., Hirst, A.G. (2010) When Microscopic Organisms Inform General Ecological Theory Ed: Woodward, G.*Integrative Ecology: From Molecules to Ecosystems Book Series: Advances in Ecological Research* **43,** p. 45-85

Reuman, D. C. and Cohen, J. E. (2004) Trophic links' length and slope in the Tuesday Lake food web with species' body mass and numerical abundance. *Journal of Animal Ecology* **73**, 852-866

Reuman, D.C., Mulder, C., Raffaelli, D., and Cohen, J.E. (2008) Three allometric relations of population density to body mass: theoretical integration and empirical tests in 149 food webs. *Ecology Letters* **11**, 1216-1228

Ricci, C. and Balsamo, M. (2000) The biology and ecology of lotic rotifers and gastrotrichs *Freshwater Biology* **44,** 15-28

Robertson, A.L., Rundle, S.D.and Schmid-Araya, J.M. (2000a) An introduction to a special issue on lotic meiofauna *Freshwater Biology* **44,** 1-3

Robertson, A.L., Rundle, S.D.and Schmid-Araya, J.M. (2000b) Putting the meio- into stream Ecology: current findings and future directions for lotic meiofaunal research *Freshwater Biology* **44,** 177-183

Rockström, J., W. Steffen, K. Noone, Å., Persson, F.S. Chapin III, E.F. Lambin, T.M. Lenton, M. Scheffer, *et al.* (2009a) A safe operating space for humanity *Nature* **461**, 472–475

Rockström, J., W. Steffen, K. Noone, Å Persson, F.S. Chapin III, E.F. Lambin, T.M. Lenton, M. Scheffer, *et al.*(2009b) Planetary boundaries: Exploring the safe operating space for humanity *Ecology and Society* **14**: 32.

Sabatino, A.D., Gerecke, R.and Martin, P. (2000) The biology and ecology of lotic water mites (Hydrachnidia) *Freshwater Biology* **44,** 47-62

Sands, C.J., Mcinnes, S.J., Marley, N.J., Goodall-Copestake, W.P., Convey, P. and Linse, K. (2008) Phylum Tardigrada: an "individual" approach *Cladistics* **24,** 861-871

Savage V.M, Gillooly J.F, Brown J H, Ist G.B, Charnov E. L., (2004) Effects of Body Size and Temperature on Population growth *Am. Nat.* **163,** No. 3, 429-441

Schmid-Araya, J.M. (1994) Temporal and spatial distribution of benthic microfauna in sediments of a gravel streambed *Limnology and Oceanography*, **39**, 1813–1821

Schmid-Araya, J.M. (1997) Temporal and spatial dynamics of meiofaunal assemblages in the hyporheic interstitial of a gravel stream. In: Groundwater/Surface Water Ecotones: Biological and Hydrological Interactions and Management Options (Eds J. Gibert, J. Mathieu and F. Fournier), pp. 29–36 *Cambridge University Press*, Cambridge

Schmid-Araya, J.M., Hildrew, A.G., Robertson, A.L., Schmid, P.E. and Winterbottom, J. (2002) The importance of meiofauna in food webs: evidence from an acid stream *Ecology* **83**, 1271–1285

Schmid, P.E., Schmid-Araya, J.M. and Tokeshi, M. (2000) Relationship between population density and body size in stream communities *Science*, **289**, 1157–1160

Sherr, E.B. and Sherr, B.F. (1994) Bacterivory and Herbivory: Key Roles of Phagotrophic Protists in Pelagic Food Webs *Microb. Ecol.* **28,** 223-235

Southward, A.J., Langmead, O., Hardman-Mountford, N.J., Aiken, J., Boalch, G.T., et. al. (2005) Long-term oceanographic and ecological research in the Western English Channel *Adv. Mar. Biol.* **47,** 1-105

Stead, T.K., Schmid-Araya J.M. and Hildrew, A.G. (2003) All creatures great and small: patterns in the stream benthos across a wide range of metazoan body size *Freshwater Biology* **48,** 532–547

Stead, T.K., Schmid-Araya, J.M. and Hildrew, A.G. (2005) Secondary production of a stream metazoan community: does the meiofauna make a difference? *Limnology and Oceanography* **50**, 398–403

Steffen, W., Crutzen, P. J., and McNeill, J. R. (2007) The Anthropocene: are humans now overwhelming the great forces of nature *AMBIO: A Journal of the Human Environment* **36**, 614-621

Swan, C.and Palmer, M.A. (2000) What drives small-scale spatial patterns in lotic meiofauna communities? *Freshwater Biology* **44,** 109-121

van der Have, T. M., and de Jong, G. (1996) Adult size in ectotherms: temperature effects on growth and differentiation *Journal of Theoretical Biology* **183,** 329–340

von Bertalanffy, L. (1960)Principles and theory of growth *In Fundamental aspects of normal and malignant growth. Elsevier, New York. Ed. Nowinski, W.W., pp. 137-259*

Walters, R.J. and Hassall, M., (2006) The temperature-size rule in ectotherms: may a general explanation exist after all? *Am. Nat.* **167**: 510–523

Walther, G.R., Post, E., Convey, P., Menzel, A., Parmesan, C., Beebee, T.J.C., Fromentin, J.M., Hoegh-Guldberg, O. and Bairlein, F. (2002) Ecological responses to recent climate change *Nature* **416,** 389-395

Walther, G.R. (2010) Community and ecosystem responses to recent climate change. *Philosophical Transactions of the Royal Society B: Biological Sciences* **365**, 2019-2024

Warwick, R.M. (1984) Species size distributions in marine benthic communities *Oecologia (Berlin)* **61,** 32-41

West, G.B., Brown, J.H., Enquist, B.J., (1997) A general model for the origin of allometric scaling laws in biology*Science,* **276**, 122-126

Winder, M., Reuter, J.E. and S. Geoffrey Schladow, S.G. (2009) Lake warming favours small-sized planktonic diatom species *Proc. R. Soc. B.* **276**, 427-435

Woodward, G., Jones, J.I.and Hildrew, A.G. (2002) Community persistence in Broadstone Stream (UK) over three decades*Freshwater Biology*, **47,** 1419-1435

Woodward, G., Ebenman, B., Emmerson, M., Montoya, J.M., Olesen, J.M., Valido, A., Warren, P.H. (2005) Body size in ecological networks*Trends in Ecol. Evol.* **20**, 402-409

Woodward, G. (2009) Biodiversity, ecosystem functioning and food webs in fresh waters: assembling the jigsaw puzzle *Freshwater Biology*, **54**: 2171–2187

Woodward, G., Benstead, J. P., Beveridge, O. S., Blanchard, J., Brey, T., Brown, L. E., Cross, W. F., Friberg, N., Ings, T. C., Jacob, U., Jennings, S., Ledger, M. E., Milner, A. M., Montoya, J. M., O'Gorman, E. J., Olesen, J. M., Petchey, O. L., Pichler, D. E., Reuman, D. C., Thompson, M. S. A., Van Veen, F. J. F., and Yvon-Durocher, G. (2010a) Ecological networks in a changing climate. *Advances in Ecological Research: Ecological Networks,* ***42***, 71-138

Woodward, G., Dybkjaer, J.B., Olafsson, J.S., Gislason, G.M., Hannesdottir, E.R., and Friberg, N. (2010b) Sentinel systems on the razor's edge: effects of warming on Arctic geothermal stream ecosystems *Global Change Biology* **16,** 1979-1991

Woodward, G.*,* Perkins. D.M., Brown, L., (2010c) Climate change and freshwater ecosystems: impacts across multiple levels of organisation *Phil. Trans. R. Soc. B***. 365:1549,** 2093-2106

Yoshida, T. (2005) Toward the understanding of complex population dynamics: Planktonic community as a model system *Ecol. Res.* **20**, 511–518

Yozzo, D.J. and Smith, D.E. (1995) Seasonality, abundance, and microhabitat distribution of meiofauna from a Chickahominy River, Virginia tidal freshwater marsh *Hydrobiologia* **310,** 197-206

Yvon-Durocher,G., Jones,J.I., Trimmer,M., Woodward,G., Montoya,J.M. (2010a) Warming alters the metabolic balance of ecosystems *Phil. Trans. R. Soc. B* ***365,*** *2117–2126*

Yvon-Durocher, G., Trimmer, M., Woodward, G., and Montoya, J.M. (2010b) Warming alters the size spectrum and the distribution of body size in aquatic ecosystems. *Global Change Biol.***17**, 1681-1694

Zachos, J., Pagani, M., Sloan, L., Thomas, E., Billups, K. (2001)
Trends, Rhythms, and Aberrations in Global Climate 65 Ma to Present *Science,* **292**, 686

Chapter 2

Study Sites and General Methods

2.1 Field Mesocosm experiment

Three of the four data chapters (*chapters 3-5*) make use of the same long-term, field mesocosm experiment located at the Freshwater Biological Association River Laboratory, (2°10`W, 50°13`N) East Stoke, Dorset, UK, which was set up by Dr. Jose Montoya, as part of a NERC fellowship (NE/C002105/1), in December 2005. Since the mesocosms were established, a number of studies have been published, highlighting the effects of warming on whole ecosystem respiration and primary productivity (Yvon-Durocher *et al.* 2010), and on benthic macroinvertebrates community structure (Dossena *et al.* 2012). See *chapter 1* of this thesis for a review of these studies in relation to this thesis. The original research presented in my thesis is focused on a 12-month period of intensive sampling of the microbial-meiofaunal loop, which I undertook on a monthly basis from February 2009 to January 2010.

The mesocosm experiment consisted of 20 outdoor, artificial ponds, each holding approximately 1m^3 water. Ten of the ponds were warmed between 3-5 °C (mean 4 °C), in line with global warming predictions for the next century, under scenario A1B (IPCC 2007) for temperate latitudes. Treatments were arranged in a randomised block design (five blocks of four mesocosms) such that each block contained two replicates of each treatment (Figure 2.1).

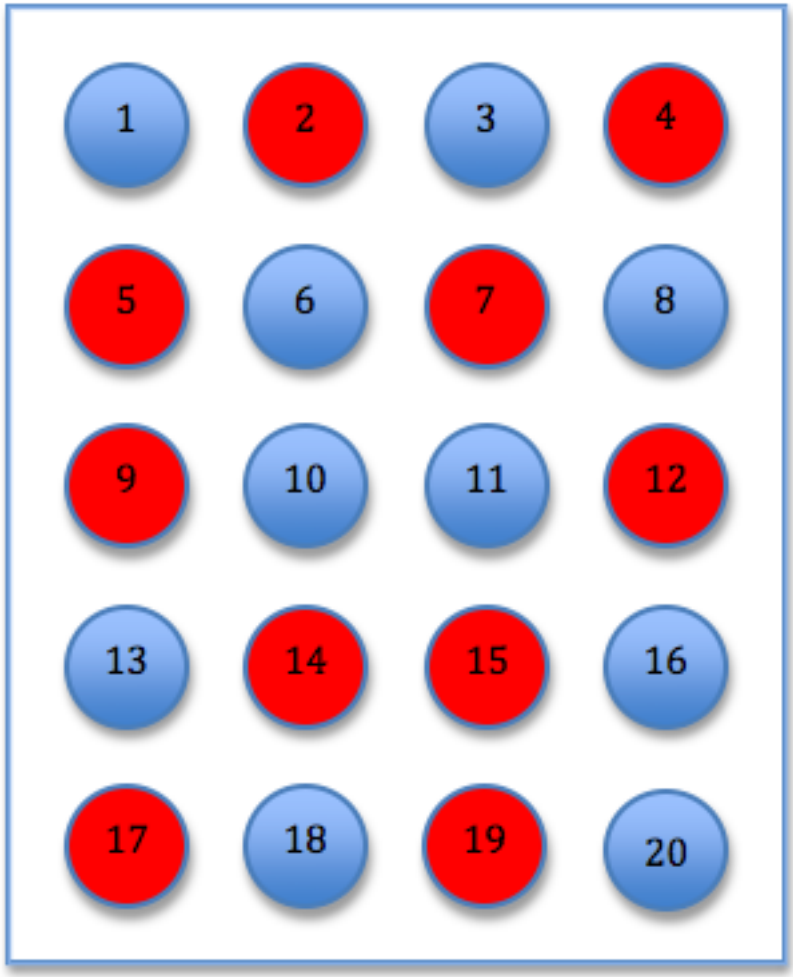


Figure 2.1 Schematic diagram of the paired design of the mesocosm experiment. Red circles indicate warmed ponds, blue ponds indicate ambient mesocosms.

Warming was achieved by an electronic heating element buried under the sediment and connected to a thermocouple that monitored the temperature in a given heated and unheated treatment pair of mesocosms (Figure 2.2 and Table 2.1).

Table 2.1 Summary of the temperature differences between heated and unheated treatments over the course of the experiment (February 2009-January 2010).

Mesocosm Pair	Mean Temperature Difference (°C)	± SE
1+2	3.764	0.013
3+4	4.314	0.014
5+6	3.526	0.012
7+8	3.802	0.015
9+10	4.8	0.013
11+12	3.164	0.011
13+14	3.9	0.014
15+16	4.210	0.012
17+18	3.840	0.016
19+20	4.132	0.017
Overall Mean	3.945	0.012

The mesocosms were seeded (in 2005) with sediment and a suite of organisms, representing an interconnected pelagic and benthic community drawn from a range of water bodies within the regional species pool from the river Frome, Dorset, UK (Yvon-Durocher et al. 2010a,b). This community contained representative species from primary producers (phytoplankton, macrophytes) [see *appendix 1*] to vertebrate predators (Roach, *Rutilus rutilus*), and a range of intermediate invertebrate consumers (Zooplankton, including *Daphnia spp.* and *Bosmina spp.*, and benthic macro- invertebrates, including Mollusca, Malacostraca, Trichoptera, Ephemeroptera and Odonata, to mimic the organismal composition (see taxa lists in *appendices1-4*), trophic complexity, and physical structure of natural pond ecosystems (Jones *et al.* 2002; McKee *et*

al. 2003). The biota was left to establish for 10 months prior to experimental warming, to allow further natural colonisation for at least one generation for the largest macroinvertebrates, and for 10s-100s of generations for the microbial assemblage (Yvon-Durocher *et al.* 2010a,b).

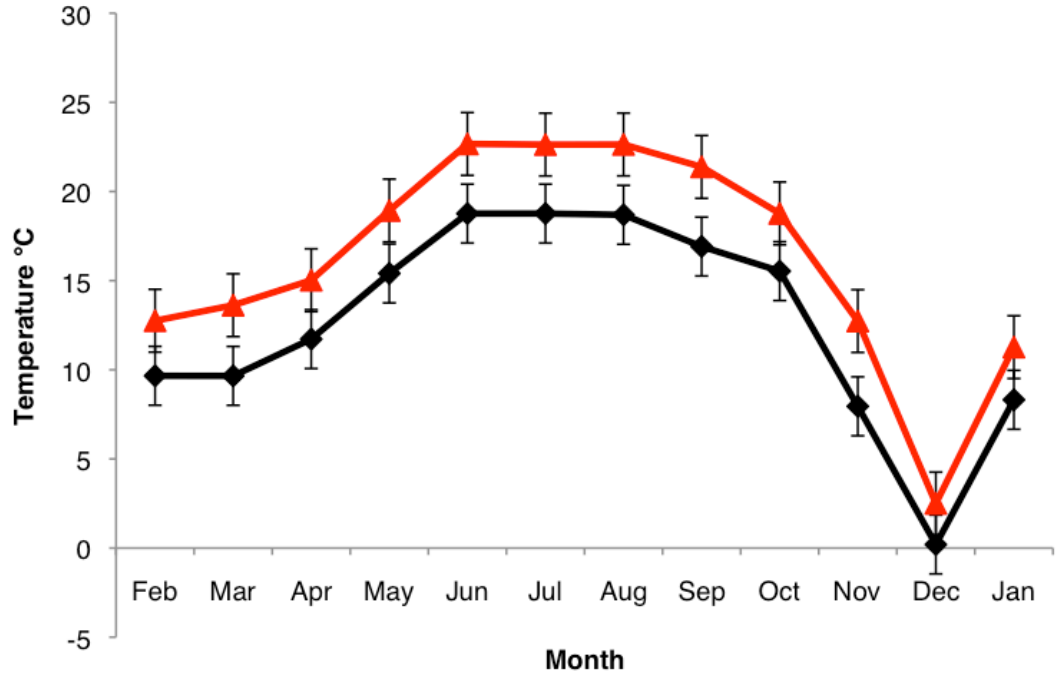


Figure 2.2 Mean temperature each month in ambient (black) and warmed (red) ponds across the sampling period for this study (February 2009-January 2010). Warmed ponds were maintained at 3-5 (mean 4) °C above the ambient ponds throughout the experimental period, from 2006 to date.

2.2 Sampling the Microbial-meiofaunal Community

Sampling of the microbial-meiofaunal assemblage in the mesocosms commenced in February 2009 and was performed monthly up until and including January 2010. To avoid pseudo-replication, ponds were treated as replicates. On each sampling occasion, a 10-ml sample was removed from each mesocosm using a sterile syringe and at three depths in the water column

(surface, mid-column and sediment) to account for the expected spatial gradient in the analysis; the surface (approximately 1cm below the surface), the mid column (defined by measuring the depth of the pond with a metre rule) and the sediment surface (approximately 2cm-5cm of sediment using a modified corer). Immediately after collecting samples from a pair of ponds, sub-samples were removed and identification and analysis was carried out (see below). Upon completion of sampling from all 10 pairs of ponds, the whole sampling procedure was repeated, resulting in a total of 120 samples (i.e. 3 x 20 x 2) each month.

The depths of the ponds (length of the water column) varied with season, e.g. levels dropped during summer due to reduced rainfall. In the event of drought, all ponds were replenished using rainwater collected previously in water butts in order to prevent drying out and cessation of the experiment.

Surface

A 10-ml syringe was used to extract 10-ml of surface water from the centre of the mesocosm, defined using a large wooden crosspiece, which was placed on the mesocosm (see Figure 2.3 a.). The 10-ml sample was transferred from the syringe to a labelled, sterile centrifuge tube for use in the laboratory. Time between sample collection and identification/counting in the laboratory was kept below a maximum of 1 hour to ensure that the community sample remained relatively unchanged, i.e. to minimise any bacterial growth/doubling of asexual species. All identification was carried out on live 1-ml sub-samples, due to the time constraints associated with processing larger numbers of samples and to

avoid logistic issues encountered when fixing these small organisms, with particular refernce to the protozoa (Corliss 1994).

Mid-column

To ascertain the approximate location of the mid-column, the length (in cm) of the water column was measured using a 1-metre rule, placed in the centre of each mesocosm. A 10-ml syringe was attached to a sampler (Figure 2.3b) at the corresponding mid-column depth per pond. A 10-ml sample was extracted and transferred to a labelled, sterile centrifuge tube for use in the laboratory. The sampler was rinsed with fresh water after each sample was taken, before being placed in the next pond, to avoid contamination between ponds.

Sediment

A mini-corer was modified from a 10-ml syringe by removing the tip and plunger of the syringe, giving a total volume of 10-ml and a diameter of 10mm. At the centre of each pond, the corer was submerged and a 10-ml core of the sediment was extracted and placed in a labelled, sterile centrifuge tube for sub-sampling and live identification in the laboratory.

In the laboratory, a 1-ml subsample was removed from each of the pond samples individually (sediment, mid-column and surface) and transferred to a 1-ml Sedgwick-Rafter counting cell for live identification using light microscopy.

a)  b) 

Figure 2.3 (a) Cross pieces placed on the mesocosms to determine the approximate centre of each pond from which samples were taken and (b) the specially designed mid-column sampler, with high-resolution centimetre scale and syringe attached.

2.3 Protist and Meiofauna Identification

All samples were examined under light microscopy using an Olympus BX50 microscope at 400x magnification (Olympus Optical, Tokyo, Japan) (Finlay and Esteban 1998). Organisms present in the pondswere identified live within a 1-ml Sedgwick-Rafter counting cell. The following comprehensive keys were used for identification of ciliates to genus in most cases: Kahl (1930, 1931, 1932, 1935); Bick (1972); Corliss (1979); Curds, Gates and Roberts (1982, 1983); Foissner *et al.* (1991); Foissner, Berger and Kohmann (1992, 1994); Foissner *et al.* (1995). For desmids, Lind and Brook (1980) was used as a comprehensive key for most known British species. In some cases it was possible to identify down to species level. For diatoms, identification was, in most cases, down to genus using Barber and Haworth (1981). For all meiofauna (including nematodes, microcrustacea etc.), identification was generally down to phyla only (largely due to time constraints of processing large numbers of samples per month), except for rotifers, where it was possible to identify down to genus and sometimes species level using Pontin (1978). Individuals were not isolated or fixed (which would have allowed samples to be characterised to species level) due to time constraints and out of a necessity to maximise sampling on each occasion.

Live individuals were photographed and individual body sizes were measured using image analysis software ImageJ (NIH, Bethesda) and Q Capture (QImaging, Surrey, BC) under light microscopy using a Nikon SMZ-U stereomicroscope (Nikon Corporation, Tokyo, Japan). Individual body dimensions (length and width) were converted to specific biovolume using common geometric formulae [see *appendix 5*] (Hillebrand *et al.* 1999). The

biovolume of the protists (ciliates and flagellates) was converted into carbon content assuming 0.14-pgC per μg^3 (Putt and Stockner 1989; Reiss and Schmid Araya 2008). Meiofaunal biovolume was converted into individual body mass by assuming a specific gravity of 1.1 and individual carbon content was estimated assuming a dry/wet weight ratio of 0.25 and a dry carbon content of 40% (Feller and Warwick 1988; Reiss and Schmid-Araya 2008).

To construct community size spectra using abundance data and individual body mass data, the total range of $\log_{10}$ (mass) values were divided into 8 bins of equal width. The same bins were used for all ponds. In each bin, $\log_{10}$ (mass) values of total population abundance of all organisms were regressed against the bin centres (Reuman *et al.* 2008; White *et al.* 2008) using $\log (N_i) = b * \log (M_i) + a$, where N_i is the abundance of the size class i and M_i is the mass at the centre of the ith size bin, b and a are the slope and the intercept respectively. The slope of the linear model (see *chapters 3-5*) describes how quickly the abundance of individuals declines with increasing mass, in the size spectrum and provides information about the community structure (White *et al.* 2007; Reuman *et al.* 2008; *chapter 1 and 3* of this thesis).

2.4 Laboratory Microcosm Experiment

In *chapter 6* I used data from microcosm experiments carried out in protist laboratories at The University of Sheffield, Department of Animal and Plant Science, from May until July 2010. Controlled-temperature rooms and water baths were used to maintain pure cultures of protists at 7 different temperatures (10, 12.5, 15, 16, 18.5, 20, 25 °C), in 250-ml microcosms containing 100-ml of

sterilised growth medium. Population density estimates were made in the laboratory, using standard aseptic techniques (Burlage *et al.* 1998).

The culture medium was Chalkley's (Tompkins *et al.* 1995) with 0.55 g/l "protist pellet", which provided a source of organic nutrients (Carolina T.M. Protozoan pellets, Burlington, NC, USA). The medium was added to one litre of water and autoclaved (>121 °C, 20 lbs/sq.in) for 4-20 minutes before adding the protists. This ensured that the experimental populations would contain only the study species and no other bacteria or protist species. Each 250-ml microcosm, containing 100-ml of sterilised Chalkley's growth medium, was inoculated with 30ml of a pure culture of one of three ciliate species; *Blepharisma japonicum*, *Paramecium caudatum* and *Tetrahymena pyriformis* from uncontaminated populations that were maintained in the same laboratory for experimental purposes. These genera were selected for comparative purposes, because they were also present in the mesocosm experiment sampling (see *chapter 6* of this thesis). Beveridge *et al.* (2010) used the same laboratory population of *Paramecium caudatum* to assess the impact of warming on swimming speed and rate of predation on a population of *Colpidium glaucoma* so, for the purpose of this study, the cultures were already well established and productive lines. (Carolina T.M. Protozoan cultures, Burlington, NC, USA).

The protists were added to the cooled, sterile media at a density of approximately 4 individuals per ml. Density was estimated using a Nikon SMZ400 stereomicroscope, petri dishes and a mass balance; 10 drops of medium containing the protists were placed on a petri dish using sterile, glass Pasteur pipettes. The mass of the 10-drops was measured

on a zeroed mass-balance and individuals per drop were counted and density recorded.

2.5 Statistical data analysis

All data analysis was performed in R Version 2.15.1 (R Development Core Team, 2012) using supplementary packages (further details are provided in individual chapters). Multivariate ordinations (PCA and RDA in *chapter 4*) were performed in CANOCO for Windows (Version 4.6).

In *chapter 3*, analysis of the community size spectra was carried out using linear mixed effects model testing for differences in the concentration of inorganic nutrients between heated and ambient mesocosms. A linear mixed effects model was conducted with restricted maximum likelihood methods using the *lme* (linear mixed-effects model) function in R (R Development Core Team 2012). Treatment (heated or unheated) was the fixed effect, and temporal pseudo-replication from repeated sampling of the mesocosms over the year was accounted for by including mesocosm identity nested within sampling occasion as random effects.

In *chapter 4*, community abundance data was first explored using multivariate ordination analysis carried out in CANOCO for windows. Secondly, three-way repeated measures ANOVAs (RMANOVAs) were performed to investigate the effect of temperature on the abundance and biomass of all groups of microbial-meiofaunal taxa as well as the potential three-way interaction between temperature, season and depth at which the samples were taken.

In *chapter 5*, the effect of warming on the abundance and body mass of 4 natural populations of protists in the mesocosms was examined. In each of the major taxa examined in *chapter 4*, the most abundant populations were chosen: 1 desmid genus, *Closterium spp.*, 1 flagellate genus *Peridinium spp.*, 1 ciliate, *Halteria spp.* and 1 population of rotifer genus (*Keratella spp.*). For individual body mass, the data from the three samples at each depth in mesocosms (*chapter 2*) were pooled and individual body mass was averaged across the mesocosms to obtain one value for each taxon per pond. Linear mixed effects (lme) models were applied to the data for each taxon to discern firstly, the effect of temperature on individual body mass and secondly, the effect of possible two-way interactions (treatment x month) on individual body size. The numerical abundance and biomass of each population were compared between treatments by repeated measures analysis of variance (RMANOVAs). The raw data were log-transformed to meet assumptions of normality and homogeneity of variance. The RMANOVAs were performed separately at each depth. Subseqeuently, depth was added into the model to test for potential three-way interactions between (i.e. treatment x month x depth).

In *chapter 6*, polynomial regression was first used on population density data for each population, to determine whether temperature had a significant effect on the rate of population decline and the rate of body mass decline. Secondly, non-linear least squares analysis was performed to approximate the model by a linear one. Finally, linear mixed effects models were used to compare different models that have been previously described in the literature for investigation of biological rates, namely allometric, complex, exponential and

Arrhenius models [(Belehradek 1926, O'Connor et al. 2007, Campbell et al. 2001, Cossins and Bowler 1987) and see *chapter 6*].

2.8 References

Barber, H.G. and Haworth, E.Y. (1981) A Guide to the Morphology of the Diatom Frustule, with a Key to the British Freshwater Genera, *Freshwater Biological Association, Cumbria*

Beveridge, O.S., Humphries, S., and Petchey, O.L. (2010) The interacting effects of temperature and food chain length on trophic abundance and ecosystem function *J. Anim. Ecol.* **79**, 693–700

Corliss, J.O. (1994) An interim utilitarian (user-friendly) hierarchical classification and characterization of the protists *Acta Protozoologica*, **33,** 1-51

Dossena, M., Yvon-Durocher, G., Grey, J., Montoya, J.M., Perkins, D.M., Trimmer, M., and Woodward, G. (2012) Warming alters community size structure and ecosystem functioning*Proc. R. Soc. B.* **279**, 3011-3019

Foissner W., Blatterer H., Berger H. and Kohmann F. (1991a) Taxonomische und o kologische Revision der Ciliaten des Saprobiensystems – Band I: Cyrtophorida, Oligo- trichida, Hypotrichia, Colpodea. Informationsberichte Des Bayerischen Landesamtes fu r Wasserwirtschaft, 1, 1–478.

Foissner, W. (1991b) Basic light and scanning electron microscopic methods for taxonomic studies of ciliated protozoa. *European Journal of Protistology*, **27**, 313–330.

Foissner W., Berger H. and Kohmann F. (1992) Taxono- mische und o kologische Revision der Ciliaten des Saprobiensystems – Band II: Peritrichia, Heterotrich- ida, Odontostomatida. Informationsberichte Des Bayeris- chen Landesamtes fur Wasserwirtschaft, 1, 1–502

Foissner W., Berger H. and Kohmann F. (1994) Taxono- mische und o kologische Revision der Ciliaten des Saprobiensystems – Band III: Hymenostomata, Prosto- matida, Nassulida. Informationsberichte Des Bayerischen Landesamtes fu r Wasserwirtschaft, 1, 1–548

Foissner W., Berger H., Blatterer H. and Kohmann F. (1995) Taxonomische und o kologische Revision der Ciliaten des Saprobiensystems – Band IV: Gymnostomatea, Loxodes, Suctoria. Informationsberichte Des Bayerischen Landesamtes für Wasserwirtschaft, 1, 1–540.

Foissner, W. and Berger, H. (1996) A user-friendly guide tothe ciliates (Protozoa, Ciliophora) commonly used by hydrobiologists as bioindicators in rivers, lakes, and waste waters, with notes on their ecology *Freshwater Biology* **35**, 375–482

Hillebrand, H., Dürselen, C.-D., Kirschtel, D., Pollingher, U., and Zohary, T. (1999) Biovolume calculation for pelagic and benthic microalgae. *Journal of Phycology* **35**, 403-424

Jones, J.I., Young, J.O., Eaton, J.W. and Moss, B. (2002). The influence of nutrient loading, dissolved inorganic carbon and higher trophic levels on the interaction between submerged plants and periphyton*Journal of Ecology*, **90**, 12-24

Kahl, A. (1930) Urtiere oder Protozoa I: Wimpertiere oder Ciliata (Infusoria) 1. Allgemeiner Teil und Prostomata *Tierwelt Deutschlands* **18**, 1–180.

Kahl A. (1931) Urtiere oder Protozoa I: Wimpertiere oder Ciliata (Infusoria) 2. Holotricha ausser den im 1 Teil behandelten Prostomata *Tierwelt Deutschlands* **21**, 181– 398.

Kahl A. (1932) Urtiere oder Protozoa I: Wimpertiere oder Ciliata (Infusoria) 3. Spirotricha. Tierwelt Deutschlands **25**, 399–650.

Kahl A. (1935) Urtiere oder Protozoa I: Wimpertiere oder Ciliata (Infusoria) 4. Peritricha und Chonotricha.Tier- welt Deutschlands **30**, 651–886

McKee, D., Atkinson, D., Collings, S.E.*, et al.* (2003) Response of freshwater microcosm communities to nutrients, fish, and elevated temperature during winter and summer *Limnology and Oceanography* **48**, 707-722

Pontin, R.M. (1978) A Key to British Freshwater Planktonic Rotifera. *Freshwater Biological Association, Cumbria*

Reiss, J. and Schmid-Araya, J.M. (2008) Existing in plenty: abundance, biomass and diversity of ciliates and meiofauna in small streams. *Freshwater Biology* **53**, 652-668.

Reuman, D. C., Mulder, C., Raffaelli, D., and Cohen, J. E. (2008) Three allometric relations of population density to body mass: theoretical integration and empirical tests in 149 food webs *Ecology Letters* **11**, 1216-1228

Reuman, D. C., Mulder, C., Banasek-Richter, C., Blandenier, M.-F. C., Breure, A. M., Den Hollander, H., Kneitel, J. M., Raffaelli, D., Woodward, G., and Cohen, J. E. (2009) Allometry of body size and abundance in 166 food webs. *Advances in Ecological Research*, **41**, 1-44

Tompkins, J., Deville, M.M., Day, J.G. and Turner, M.F. (1995). Culture Collection of Algae and Protozoa: *Catalogue of Strains 1995. Kendal: Titus Wilson and Son Limited*

White, E.P., Enquist, B.J. and Green, J. (2008) On estimating the exponent of power-law frequency distributions *Ecology*, **4**, 905–912

Chapter 3

Size spectra and allometries of microbial-meiofaunal assemblages in experimentally warmed mesocosms

3.1 Abstract

Ascertaining the impacts of global change, such as rising temperatures, as a result of anthropogenic activity, on the structure and functioning of the microbial compartments and processes that drive many ecosystem processes is a major challenge in the field of ecology.

I conducted a long-term, intergenerational, freshwater mesocosm experiment designed to assess the impacts of simulated global warming on microbial loop organisms within the food web. Abundance and biomass of individuals was recorded, and size spectra of "microbial loop" organisms constructed over 12 months from 20 mesocosms (10 warmed to ~3-5 °C above ambient and 10 ambient, as a control). The abundance and body mass of 5 major taxa was recorded; the single celled algae (desmids and diatoms), autotrophic and heterotrophic flagellates, ciliates, amoebae and all permanent, microscopic metazoans (meiofauna). I tested the following hypotheses; (1) Warming will result in a steeper slope of the microbial community size spectrum which corresponds to a prevalence of smaller individuals at warmer temperatures, (2) the effect of warming on microbial assemblages will be most pronounced within the autotrophic organisms (i.e. desmids, flagellates), (3)

warming will reduce average individual body mass, as a result of the temperature size rule (TSR) and (4) Warming will reduce total community biomass, in line with the metabolic theory of ecology (MTE) due to elevated metabolic rates of individuals placing a higher energetic demand on the environment.

Results showed that the slope of the size spectrum of the whole microbial community was not significantly different between warmed and ambient treatments. The relative abundance of heterotrophs was significantly greater over the whole sampling period, in warmed ponds compared to ambient ponds but total biomass was similar between autotrophs and heterotrophs, indicating that individuals may be smaller overall in warmed mesocosms compared to ambient. The size spectrum for the whole community was non linear, giving a multi-modal appearance, indicating that these taxa do not follow a power law function as has been observed for many other taxa.

Shifts in the total biomass and abundance between functional groups (i.e. increased abundance of heterotrophs compared to autotrophs has consequences for the functioning of aquatic systems in a warmer world.

3.2 Introduction

The planet is warming at unprecedented rates, with the surface temperature in temperate regions predicted to rise by 3-5°C in the next century, under scenario A1FI, of the IPCC report (2007). This increase is largely attributable to anthropogenic activity as Earth enters the Anthropocene era with elevated greenhouse gas emission, deforestation and habitat destruction etc., as the human population continues to increase (Miller *et al.* 2005; Steffen *et al.* 2007). Understanding how human driven climate change impacts natural communities and the implications of this is a complex and crucial challenge for contemporary ecologists. Recent and ongoing research highlights how warming impacts at every level of organisation in both natural (e.g. Jacobsen *et al.*1997; Walther 2010; O'Gorman *et al.*, 2012) and artificial (e.g. Petchey *et al.* 1999; Yvon-Durocher *et al.* 2011) systems at the individual, population, community and whole ecosystem level. Parmesan (2006) describes how climate change has had impacts on every continent and every environment across the globe and within every major taxonomic group.

The body size of an individual is recognised as a key ecological feature because it affects an organism's biology (including life history, physiology, behaviour and ecology) (Peters 1983, Brown *et al.* 2004, Woodward *et al.* 2005, White et al. 2007, Sibly et al. 2012). Body size is related to life history by the simple power law:

$$\mathbf{Y = a\ W^{b}}.$$

Where Y is the life history trait or physiological process in question, 'W' is the body mass of the individual, **'a'** is the intercept and **'b'** is the scaling exponent.

The relationship between body size and ecological characteristics has been useful to assess the state of, and inform the management and conservation in marine systems (Shin *et al.* 2005) and has also been used in freshwater systems to assess the impacts of global warming on the size spectra of phytoplankton zooplankton and macroinvertebrates in experimental mesocosms (phytoplankton and zooplankton: Yvon-Durocher *et al.* 2010; macroinvertebrates: Dossena *et al.* 2012). In size spectrum theory, individuals are viewed as particles (Sheldon *et al.* 1972) and the relationship between the mass of individuals and the abundance of individuals is quantified. In addition, abundance and production are often linked to individual level processes and with body mass, and this relationship originates from early observations that small species are more abundant than larger ones (Elton 1927; Lindeman 1942). This relationship is typically defined as a simple, inverse, linear correlation between body mass and abundance on a log-log axis (Damuth 1981, 1987) and is considered a powerful descriptor of how energy and nutrients are partitioned within the biomass of an ecosystem (White *et al.* 2007). This has effect has been observed in microbial compartments (including bacteria) by Cavender-Bares *et al.* (2001) who demonstrated clear power laws in the oceanic microbial assemblages they examined.

Body size, in turn, appears to be affected by temperature, where organisms tend to be larger in colder regions (Bergman 1847, Ray 1960, James 1970, Ashton et al. 2000, Ashton 2002), which suggests that global warming may alter the distribution of body sizes *via* species range shifts (Chen et al. 2011) and/or physiological adaptation (Musolin 2007). Several non-mutually exclusive explanations have been proposed to explain why warming appears to

favour the smaller body sizes (Daufresne *et al.* 2009); (1) James's Rule, which predicts that the mean body size of a species population will decline with temperature (James 1970) and (2) The Temperature-Size Rule (TSR) which is a subset of James's Rule and predicts that oxygen demands and different thermal sensitivities in growth and development rate will lead to smaller size at a given age in warmer temperatures (Atkinson 1994, Walters and Hassall 2006).

Different allometric relations of abundance to body mass are based on either individuals or species but all attempt to link community and population level patterns in abundance and body size (governed by power laws), to the metabolic needs of the individual. Studies addressing body mass–abundance relationships mainly follow two approaches when visualising the relationship on a species level: body mass of species can be plotted against population abundance or size bins (total range of $\log_{10}$ (M) values, where M is body mass in μg C, into n logarithmic bins of equal width) can be created (see the methods section of this chapter) and the abundance of similar sized species is summed per size bin (Blanco *et al.* 1994). Construction of individual size distributions (ISD), describes the distribution of individual-organism body mass, disregarding taxonomy and provides the same information as the abundance spectrum defined by Kerr and Dickie (2001) by giving information about how resources are partitioned among size classes (irrespective of taxonomy). Secondly, using species size distributions (SSD), the frequency distribution of species-level average masses is used and does not include information on abundances of the component species. In this chaper, I use ISD to examine how resources are partitioned in the microscopic, eukaryotic compartment of the microbial loop and

compare the size spectra between warmed and ambient treatments to assess potential responses of the microbial loop to global warming. The slope of the size spectrum results from the joint change in abundance and size of organisms occurring across a trophic link. Observed size spectra slopes typically become steeper (more negative) following perturbations such as environmental change (e.g. warming), exploitation (fishing) and species invasion, as larger organisms tend to be most vulnerable to disturbance (Petchey and Belgrano 2010).

In the context of the metabolic theory of ecology (MTE) (Brown *et al.* 2004), 3/4-power allometric scaling should be observed for individuals within a species when all species use the same amount of energy (and assuming that all individuals within a species use the same resources), as for the energy equivalence rule (Damuth 1987; Nee *et al.* 1991). The MTE requires that abundance of all coexisting individuals within a trophic group and body mass category should be summed because the theory predicts that this will determine how many individuals of a given size range and trophic group can be supported by a given resource (Brown *et al.*, 2004; Dinmore and Jennings, 2004). The assumption is that all individuals (MTE is for individuals) use the same resources and using data like this should be used to explicitly test the energetic equivalence rule, but for most studies, data are compiled for species rather than trophic groups (e.g. Griffiths, 1998; Schmid *et al.*, 2000). Here, I have split the microbial community data into autotrophic and heterotrophic groups to examine the scaling exponent (the slope of the Mass-abundance plot) under the assumption that the organisms within the trophic groups, organisms will use resources similarly and to examine the responses of the microbial loop in the context of size spectra theory.

Organisms of the microbial loop play a major role in the assimilation of dissolved organic carbon, making it available to the higher trophic levels (Azam *et al.* 1983; Fenchel 2008; Landry and Calbert 2004). The energy transfer efficiency from the microbial loop (protists e.g. flagellates, ciliates) to the micro-metazoans (e.g. copepods, rotifers) is thought to be low compared to the transfer efficiencies between other groups (e.g bacteria to protistan grazers; copepods to fish) because of the many trophic levels between small phytoplankton and micro-metazoans (Ducklow *et al.* 1986). Increased temperatures as a result of climate change may work to change this by either increasing efficiency of energy transfer or decoupling the transfer. Steeper slopes may also imply an increased prevalence of smaller organisms, resulting in a reordering of the biomass structure of the food web (Yvon-Durocher et al. 2011) and/or suppression of the relative abundance of large organisms (Pauly et al. 1998). Small invertebrates such as protozoans and micro-metazoans are an intriguing group in this context because they represent a transitional zone where body sizes of single-celled and multicelled organisms overlap and where many traits, such as reproduction and feeding modes, change fundamentally. Placing these small organisms in the context of allometric scaling theories might help to reveal whether there is a universal phenomenon, which applies to small and large, single and multicellular animals. However, allometric studies on microscopic organisms are sparse (but see Schmid *et al.*, 2000, 2002; Finlay 2002; Stead *et al.*, 2005; and studies on microbial size spectra, e.g. Cavender-Bares *et al.* 2001; Gasol *et al.*, 1991; Li 2002). Only a few studies have considered the protozoa or meiofauna (Finlay, 2002; Schmid *et al.*, 2000, 2002; Stead *et al.*, 2005) and found the responses to be unique to these small

organisms. On the whole, these small organisms respond differently to large organisms in terms of changing environments (for a review see Reiss *et al.* 2010) and for this reason, caution is required when extrapolating cause and correlation from microcosms and mesocosms, to higher levels of organisation e.g. to whole ecosystem level as in Yvon-Durocher *et al.* (2011).

In this study, I simulated the effect of rising global temperatures on the size spectra, total community biomass and thebiomass of heterotrophs and autotrophs separately to examine the responses of the whole community and it's composite functional groups. I make use of a long-term mesocosm experiment that has been running for several thousand generations of the study organisms, looking for a potential end point as the system moves towards new equilibria conditions after long term warming. I specifically focus on the eukaryotes; the protists (autotrophic and heterotrophic groups) and the permanent meiofauna present in the system.

Previous studies in the same mesocosm experiment showed a shift towards heterotrophy, by observation of increased ecosystem respiration compared to primary production (Yvon-Durocher *et al.* 2010a) and a shift in the size spectra of phytoplankton (Yvon-Durocher *et al.* 2011; Dossena *et al.* 2012). I investigated whether this apparent shift towards greater heterotrophy is reflected in the size spectrum of the microbial loop by including five groups of eukaryotic organisms that comprise the microbial loop; desmids, ciliates, flagellates and amoebae are included in the protozoa and all permanent meiofauna (e.g. rotifers and nematodes). I tested the following hypotheses:

(i) Warming will result in a steeper slope of the microbial community size spectrum which corresponds to a prevalence of smaller individuals at

warmer temperatures, in line with current theories that warming favours smaller individuals (e.g. Daufresne *et al.* 2009; Winder *et al.* 2009; Moran *et al.* 2010).

(ii) The effect of warming on microbial assemblages will be most pronounced within the autotrophic organisms (desmids, flagellates), in line with previous studies in the ponds (Yvon-Durocher *et al.* 2011; Dossena *et al.* 2012).

(iii) Warming will reduce average individual body mass, as a result of the temperature size rule (TSR), with individuals responding to warming by reaching a smaller size as adults (Atkinson 1994; Atkinson *et al.* 2003; Daufresne 2009; Winder et al. 2009).

(iv) Warming will reduce total community biomass, in line with the metabolic theory of ecology (MTE) due to elevated metabolic rates of individuals placing a higher energetic demand on the environment (Allen *et al.* 2002; Brown *et al.* 2004).

3.3 Methods

Experimental Design

The experiment was part of an ongoing project set up in December 2006 at the Freshwater Biological Association River Laboratory, East Stoke, Dorset, UK. A detailed description of the experimental set-up is found in Yvon-Durocher *et al.* (2010 a,b,c) and in the methods section (*chapter 2*) of this thesis provides more details about the study site. Briefly, it consisted of 20 freshwater mesocosms (~1m^3, 0.5m water depth): ten replicates were left at ambient temperature whilst the other 10 were warmed to between 3 and 5 °C (mean 4 °C) above ambient.

They were seeded with organisms from the regional species pool (river Frome), (listed in the appendices of this thesis), before warming commenced.

Sampling the Microbial Loop

The microbial loop community from each of the 20 mesocosms was sampled on a monthly basis between February 2009 and January 2010. Ten millilitre samples were taken from three levels of the water column in each mesocosm (30-ml from each pond in total); the surface (using a 10ml syringe); the mid column (at approximately 50 cm depth), or exactly half way between the surface and the sediment (see general methods for water column depths across the sampling season). Mid-column samples were taken using a specially designed sampling device, consisting of a metal frame with a one-metre scale and a syringe attached at the approximate centre of the mid-column (see *chapter 2* of this thesis). The sampling device was placed in the centre of each pond and disturbed sediment was allowed to settle before the 10-ml sample was taken using the syringe. Finally, the sediment surface was sampled using a syringe, modified to resemble a core sampler (see *chapter 2* for a full description). The three samples from each mesocosm were used separately in the analysis as 1-ml subsamples were taken were counted and recorded separately to assess the spatial distribution of individuals in the samples. All organisms present in a 1ml subsample were counted using a Sedgwick rafter cell (c.f. Finlay and Esteban 1998) and length and width measurements were taken using Q Capture Image analysis software (QCapture PRO 7).

Size bin construction

To address hypothesis (i), community size spectra (CSS) were plotted (see *chapter 2*). Size bins were constructed by dividing the total range of $\log_{10}$ (M) values, where M is body mass in μg C, into n logarithmic bins of equal width, and the logarithm of the total abundance of all organisms ($\log_{10}$N) in each bin was regressed against the centre of the bin (after White *et al.* 2008). The 'size' of all organisms was expressed as mass in units of carbon (μg C). Mass was determined by converting biovolume to fresh weight using a factor of 1.1, and carbon content was then estimated from a dry/wet weight ratio of 0.25 and a dry carbon content of 40% [see *appendix 4* (Hillebrand *et al.* 1999, Reiss and Schmid-Araya 2008)]. The slope of the linear model describes how quickly the abundance of individuals declines with increasing size in the size spectrum.

Statistical analysis

Differences between treatments (warmed and ambient) were analysed (to test each hypothesis in turn) for the following community properties: (1) the slopes of the size spectra for the whole community and for autotrophs and heterotrophs separately [(hypothesis (i) and (ii)], (2) average individual body mass (hypothesis (iii) and (3) total community biomass as well as total autotroph and heterotroph biomass and abundance (hypothesis (ii) and (iv). I performed three separate repeated measures ANOVAs (RMANOVAs) (one for each depth in each mesocosm) to test for differences between treatments and to account for temporal pseudoreplication in the data (repeated measurements in each mesocosm on twelve dates, (fitted as random effects). Variables tested *per* sampling occasion (month) and per sample depth were (i) the abundance (number of individuals in 1-ml of the Sedgwick rafter counting cell), (ii) individual body mass (μg C) [average body mass of individuals per treatment (n=10)] and

(iii) total standing biomass (μg C ml $^{-1}$) was expressed as the sum of the average individual body masses (μg C) per mesocosm multiplied by the abundance of individuals All analyses were performed using R statistical software (R Development Core Team, 2011). Abundance and standing biomass was $\log_{10}$-transformed prior to analysis to achieve normality.

3.4 Results

Groups identified

A large number of autotrophic and heterotrophic taxa were identified from the 1-ml subsamples in the ponds; broadly, including Desmids, Diatoms, Flagellates, Ciliates, Rotifers and Daphnids (see *appendices 1-3* for taxa lists). For the purpose of analysis, the groups were split broadly rather than identifying to species level, due to time and logistical constraints. Autotrophic organisms used in the analysis were confined to the microscopic algae (desmids and diatoms) and excluded prokaryotic autotrophic groups (e.g. cyanobacteria), flagellates and individuals of photosynthetic genera (e.g. *Euglena spp.* and *Peridinium spp.*, heterotrophic protists (e.g. ciliates and amoebae and the meiofauna (all permanent, multicellular, microscopic eukaryotes in the size range 500 μm-1mm (Stead *et al.* 2003; Reiss *et al.* 2008).

Whole community size spectra

Contrary to hypothesis (i) that warming will result in an overall steeper slope for whole community size spectra due to higher energetic demands, the slopes of the whole community size spectra plots for warmed and ambient ponds were not significantly different. The CSS slope for ambient was -0.369 (95% CI -0.33

to -0.39), intercept = 3.24 and for warmed mesocosms was -0.367 (95% CI -0.33 to -0.39) and intercept 3.19. The size spectra of the microbial community in both warmed and ambient ponds sampled here also appear non-linear (multi-modal) (Figure 3.1) and do not follow patterns observed in previous studies (Yvon-Durocher 2010c; Dossena *et al.* 2012).

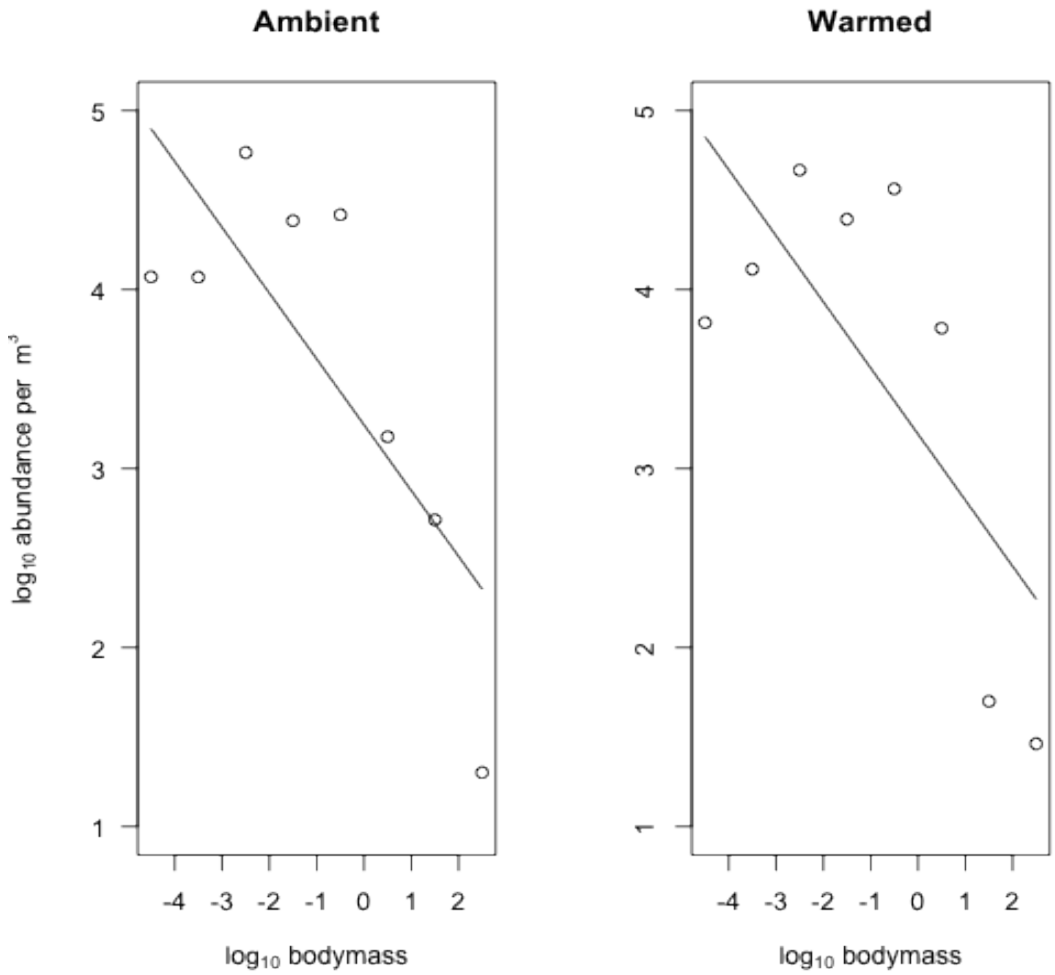


Figure 3.1 Community Size spectra (CSS) slopes for ambient (left panel) and warmed ponds (right panel) were not significantly different. The CSS slope for ambient was -0.369 (95% CI -0.33 to -0.39), intercept = 3.24 and for warmed mesocosms was -0.367 (95% CI -0.33 to -0.39) and intercept 3.19. The smallest size classes appear lower in abundance than medium sized classes, making the CSS non-linear in appearance.

Size spectra of autotrophs and heterotrophs

In support of hypothesis (ii), the separate size spectra for autotrophs and heterotrophs (Figure 3.2) showed different patterns to those of the whole community. The slope for autotrophs in ambient mesocosms= -0.44 (95% CI -0.4 to -0.47) and intercept 4.1 (95%CI 3.7 to 4.4) whereas the slope for autotrophs in the warmed mesocosms = -0.62 (95% CI -0.59 to -0.65), intercept = 3.82 (95% CI 3.3 to 4.1). This difference was significant (df=1,69, p=0.0046, F=16.74). This is in support of hypothesis (ii) that warming will result in a significantly steeper slope in autotrophic organisms and agrees with previous studies in the ponds that warming alters the size spectrum of autotrophic taxa more so than heterotrophic taxa (Yvon-Durocher *et al.* 2010c). The size spectra slopes for heterotrophs were not significantly different between treatments (Figure 3.2).

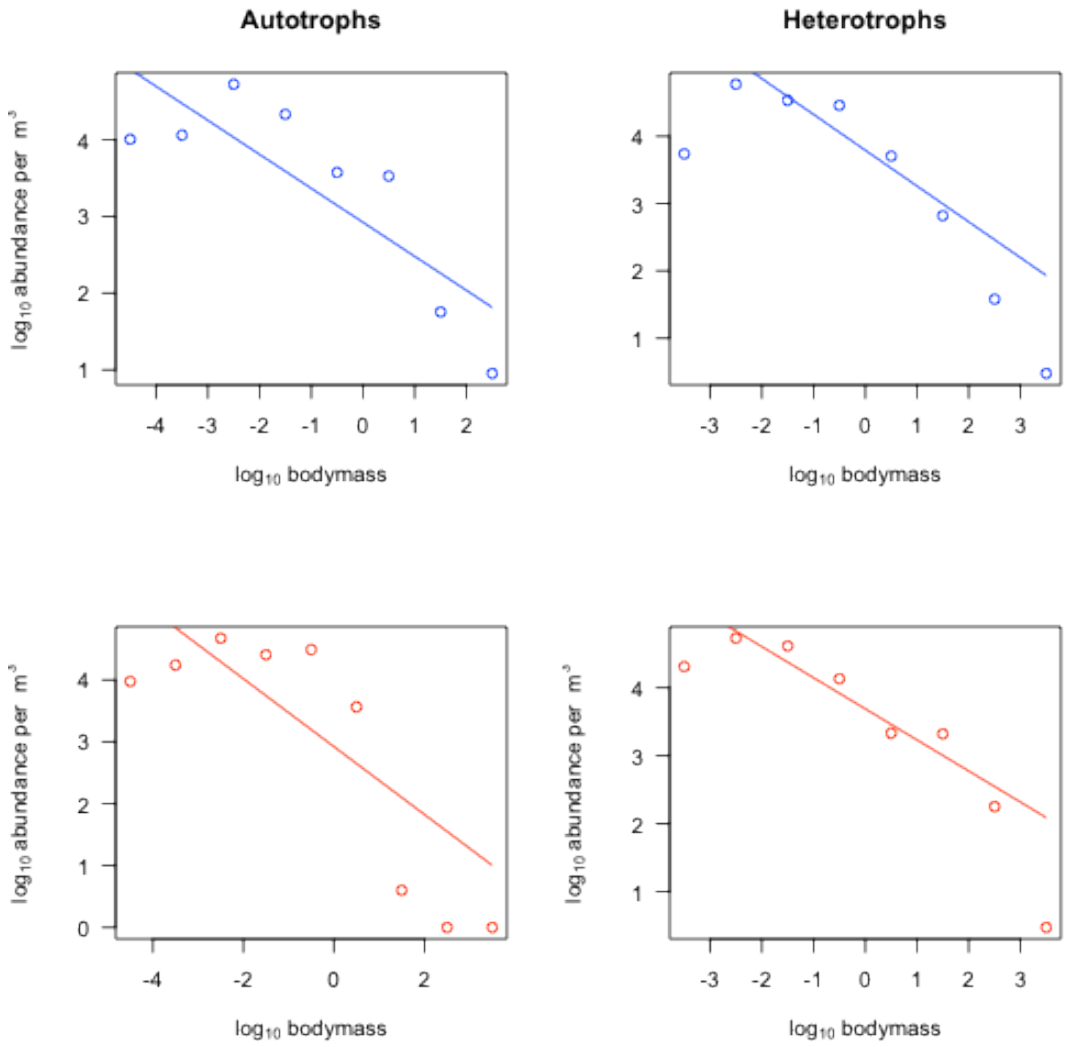


Figure 3.2 Size spectra of autotrophs (left panels) and heterotrophs (right panels). For autotrophs, the slopes of the size spectra differ significantly (df=1,69, p=0.0046, F=16.74). The slope for autotrophs in ambient mesocosms= -0.44 (95% CI -0.4 to -0.47) and intercept 4.1 (95%CI 3.7 to 4.4) whereas the slope for autotrophs in the warmed mesocosms = -0.62 (95% CI -0.59 to -0.65), intercept = 3.82 (95% CI 3.3 to 4.1).

Whole community biomass

Warming did not have a significant effect on the total community biomass over the whole sampling period (p=0.072, $F_{1,69}$=36.74). The two-way RMANOVA performed indicated a significant interactive effect of temperature with occasion

($p=0.0331, F_{1,69}=50.17$) (Table 4.1) and suggests that warming has an effect on the phenology of these small organisms.

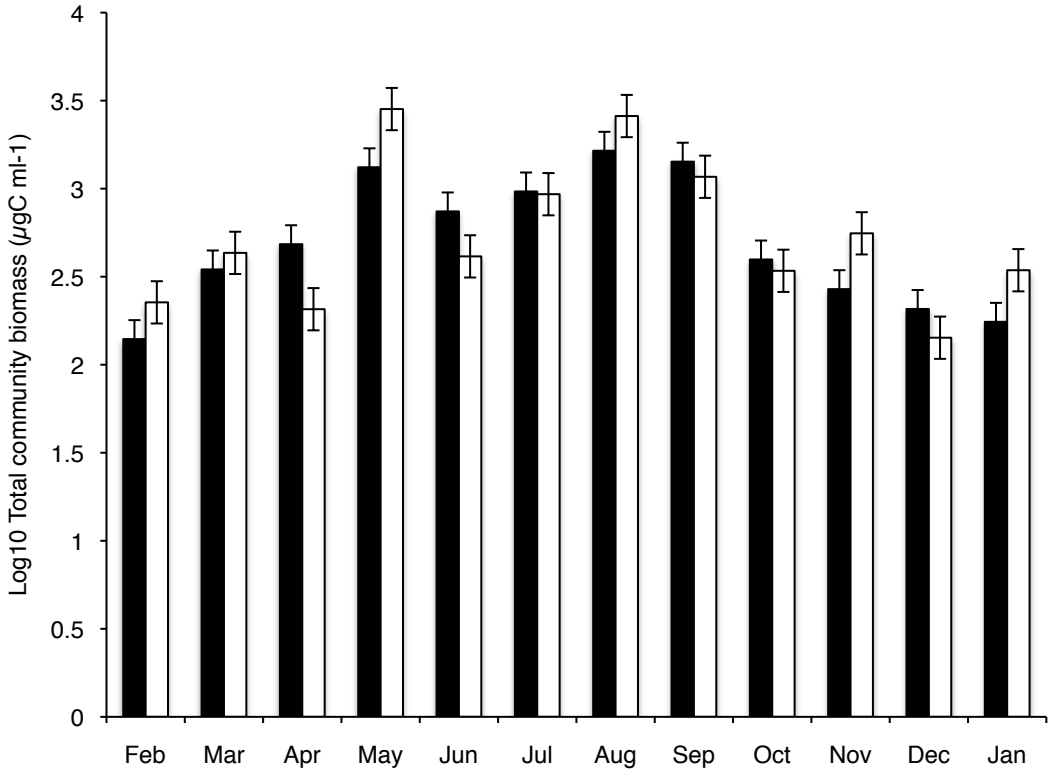


Figure 3.3 Total community biomass (μgC ml^{-1}) (±1SE), across the study period, irrespective of whether individuals are autotrophs or heterotrophs, per treatment as the mean from 20 ponds across the sampling period from February 2009 (Month1) until January 2010 (month 12). Black bars show the seasonal pattern in biomass within the ambient ponds and white bars indicate warmed ponds. ANOVA revealed treatment does not have a significant effect overall but the interaction between month and temperature is significant (p=0.04, df=1,69, F=15.1).

Table 3.1 Results of the RMANOVAs for the mean whole community biomass with all significant interaction terms of treatment by occasion and depth in the water column for the community biomass. Treatment refers to heated and ambient ponds, occasion refers to the sampling period (February 2009- January 2010) and depth refers to the point in the water column from which samples were taken.

WHOLE COMMUNITY BIOMASS			
Variable	**df**	**F-ratio**	**p-value**
Treatment	1,9	3.78	0.073
Month	1,11	9.23	**0.033**
Log biomass~ Treatment x month x depth	1,69	36.57	**0.024**
Log biomass~ Treatment x month	1,23	54.25	**0.036**
Log biomass Treatment x depth	1,4	11.8	**0.041**
Log biomass~ Month x depth	1,34	21.53	**0.021**

Autotroph and heterotroph abundance and biomass

Mean total abundance and biomass (±1SE) were averaged across all individuals per mesocosm, across all ponds per treatment (n=10), for the entire sampling period. Black bars represent ambient ponds and white bars represent warmed ponds. The abundance of both autotrophs and heterotrophs was significantly different between treatments; with elevated abundance of both shown in warmed ponds. However, the total biomass of all autotrophs and all heterotrophs were not significantly different between treatments (Figure 3.4), suggesting that, despite increased abundance at elevated temperatures, individuals may be smaller.

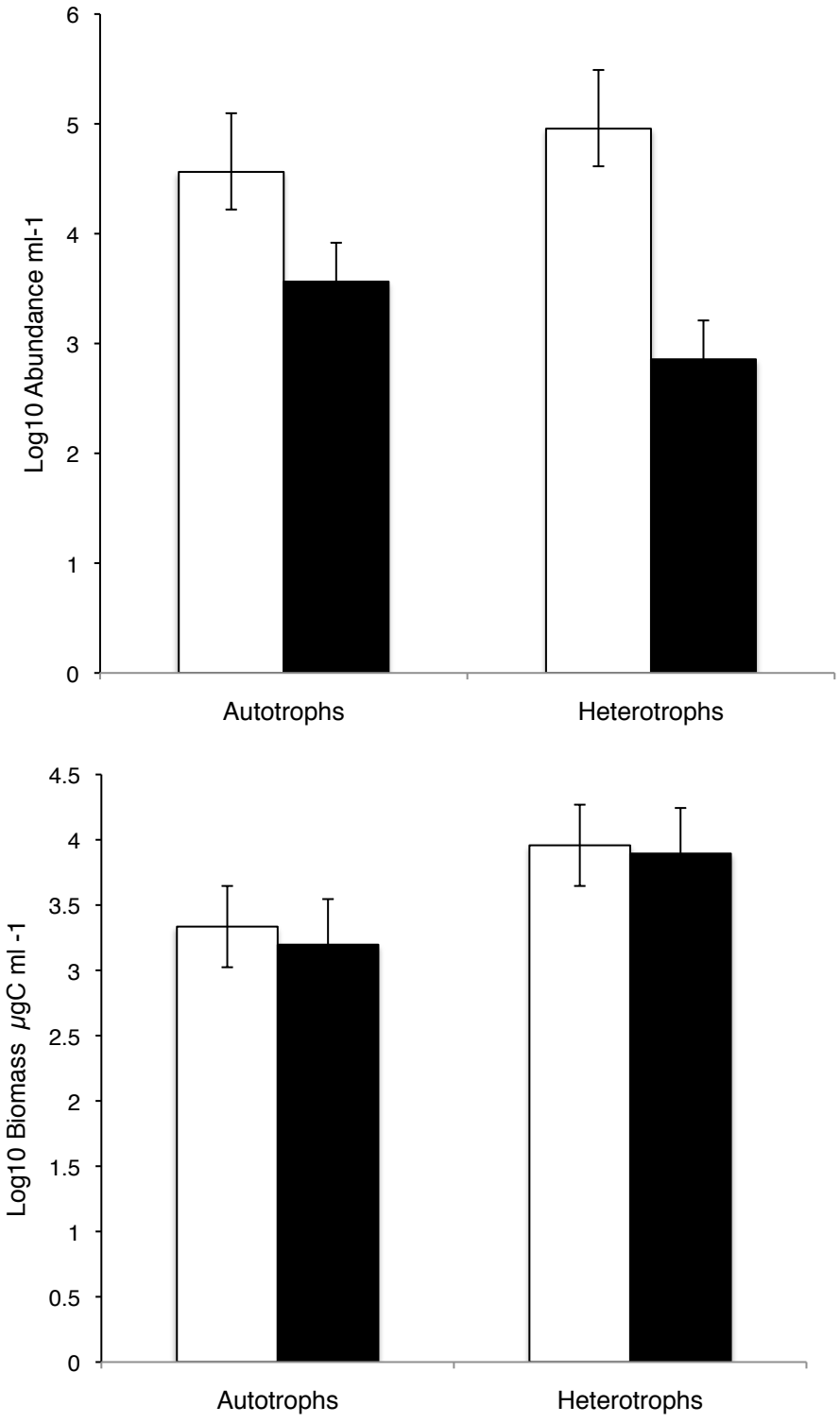


Figure 3.4. Summary totals of autotrophic and heterotrophic abundance (top panel) and biomass (bottom panel) in all 20 ponds. Mean total abundance and biomass (±1SE) were averaged across all individuals per mesocosm, across all ponds per treatment (n=10), for the entire sampling period. Black bars represent ambient ponds and white bars represent warmed ponds. Heterotrophs were significantly more abundant in warmed ponds compared with ambient ponds, but biomass was not significantly different.

Mean individual body mass

Across the whole sampling period, there was an overall significant effect of treatment on the body size of individual organisms, despite non-significant results of the size spectra; the mean individual body of all organsisms counted and measured, regardless of whether they were autotrophic or heterotropic, was larger in ambient ponds, compared to warmed ponds.

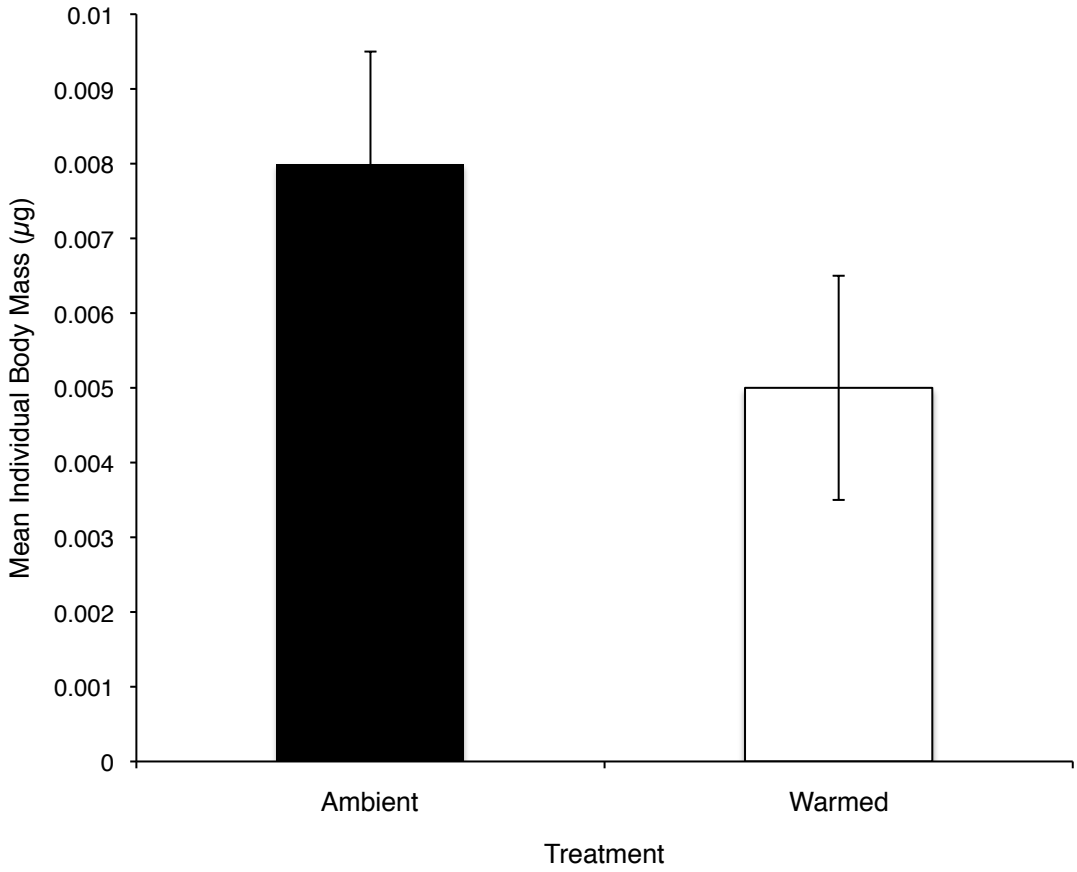


Figure 3.5 Log mean individual organism mass (μg), (±1SE), irrespective of functional group, for each treatment, averaged across all individuals per pond to get one value per mesocosm and averaged across ten mesocosms per treatment (n=10). Individuals are significantly larger in the ambient (control) ponds than in the warmed ponds. Data are presented as the mean individual mass of all organisms in the ponds, per treatment (ANOVA $F_{1,9}$=14.5, p=0.04).

3.5 Discussion

Community size spectra (CSS)

Size spectrum theory predicts that the steepness of the slope of the community size spectrum (CSS) is the product of the efficiency of energy transfer from small, abundant organisms at the base of the food web to large, scarce predators at the top. This effect has been in observed in ecological processes across multiple levels of organisation, from individuals (Peters 1983; Brown *et al.* 2004), their interactions (Emmerson and Raffaelli 2004; Berlow *et al.* 2009) to populations (Damuth 1981; Jennings and Mackinson 2003; Reuman *et al.* 2008), communities and ultimately, ecosystems (Petchey *et al.* 2008; Yvon-Durocher *et al.* 2010, 2011). Despite this widespread occurrence, in this study, warming did not result in a steeper slope for the whole community size spectrum (Figure 3.1) and hypothesis (i) that warming would result in a steeper slope, in line with classic size spectrum theory and findings from previous studies in the same systems (Yvon-Durocher *et al.* 2010c; Dossena *et al.* 2012) was therefore not supported. In addition to this absence of treatment effect, the size spectra and allometries of the main groups of organisms in the microbial loop, studied here yielded slopes which were not multiples of 0.25, contravening previous studies which attempt to apply this allometric scaling to a wide range of taxa, from microbes to large metazoans (Gillooly *et al.* 2001,2002; Brown *et al.* 2004). Instead, size spectra were non-linear, indicating that the protistan and meiofaunal community sampled does not follow a power law and hence may be fundamentally different to other eukaryotes. The possibility of protists and meiofaunal constituents not following ¼ power laws has already been suggested in previous laboratory work by DeLong *et al.* (2010) who found

slopes of protists and micro-metazoans to be much steeper than those for larger metazoans and has also been observed in a stream system (Reiss *et al.* 2010). These findings highlight that these small organisms do not behave in the same way as larger eukaryotes, under environmental perturbation.

After initial size spectra assessment, the data were split by trophic status i.e. autotrophs (obtain energy from sunlight) and heterotrophs (obtain energy from phagotrophy). For this, there was a significant difference in the slopes of the size spectra for autotrophs only. Temperature does not appear to directly impact the size spectra of heterotrophic microbial loop organisms, shown by an non-significant result as also suggested in some other studies (e.g. Aberle *et al.* 2006; Guhl, 1994), but may still have an interactive effect with other factors, e.g. with seasonal and spatial gradients (e.g. Berger *et al.* 2007). This interactive effect of temperature with other factors has been demonstrated in other studies with nutrient addition (e.g. Winder and Schlindler 2004) and with seasonal and spatial dynamics (e.g. Berger *et al.* 2007).

Hypothesis (ii) predicted that the autotrophic species would be more responsive to warming than heterotrophic species the slopes of the log-log plots of abundance versus body size show shallower slope in the warmed ponds compared to the ambient ones.

In agreement with hypothesis (iii) In this experiment, warming had a more pronounced effect at different times throughout the seasonal cycle, suggesting a more subtle phenological response that is obscured by averaging across the year (see *chapters 4 and 5*). This has also been by observed and reported by Aberle *et al.* (2006), where warming had the greatest impact on protistan communities during the winter, where warmer temperatures promote

the persistence of species that would otherwise encyst at lower, unfavourable temperatures. In addition to this, a study by Dossena *et al.* (2012) showed opposite shifts in the size spectra at the beginning and the end of the growing season (April and October respectively), of the macroinvertebrate community using the same mesocosm experiment as in this study; in April, individuals were typically smaller in warmed mesocosms compared to ambient mesocosms and in October, individuals were typically larger in warmed mesocosms, compared to ambient mesocosms and was attributed to warming and suggest that these opposite shifts may be due to an interaction of temperature with the sampling date, hinting at the likelihood of phenological shifts in the benthos.

Community biomass

Warming did not have a significant effect on the total community biomass. In contrast, the overall abundance of heterotrophs per ml of sample was significantly lower in ambient ponds compared with warmed ponds yet the total biomass of heterotrophs was comparable (Figure 3.3): i.e. individuals were on average smaller in the warmed ponds (Figure 3.4) which corroborates current theory regarding the effect of warming on ectotherms – warming is expected to favour smaller body sizes and has been described as the third universal response to climate change, alongside range shifts and changes in phenology (body size: Daufresne *et al.* 2009; Winder *et al.* 2009; Moran *et al.* 2010).

Sheridan and Bickford (2011) discuss the potential ecological implications of shrinking body size as a universal response to warming; it is likely that smaller organisms are selected for and that as overall body size reduces, species-species interactions are unaffected overall but effects may

become apparent at the individual (smaller body size than individuals in cooler environments) and at the population level but less pronounced at the ecosystem level and therefore the effect will be masked in size spectra analysis. This is also reflected in the size spectra of the community examined in this study, indicated by a lack of detectable effect of temperature at the whole community level but once functional groups are discerned (here, split into autotrophic and heterotrophic eukaryotes), the effects of warming become apparent.

At the individual level, current theories such as the temperature size rule (TSR) (Atkinson 1994; Atkinson *et al.* 2003) and the metabolic theory of ecology MTE (Gillooly *et al.* 2001, 2002; Brown *et al.* 2004) attempt to explain this apparent response to warming on a mechanistic level and have resulted in much related research in the past decade. The observed reduced body size of the microbial loop organisms in this study has potential implications for the functioning of other natural ecosystems, for instance, impacts on nutrient cycling rates, which should increase with reduced body size as larger bodied organisms retain nutrients for a longer period of time (e.g. Elser and Urabe, 1999; Brown *et al.* 2004) and hence effects at the individual level may ramify to higher ecosytem-levels through biotic interations (Montoya and Rafaelli 2010).

The TSR and MTE are not necessarily mutually exclusive and as yet, no single mechanistic explanation exists to explain this phenomenon of reduced body size and there is ongoing debate regarding the universality of such general theories. Forster *et al.* (2011a) have developed a conceptual theme to explain how multicellular and unicellular organisms achieve this change in size through different mechanisms; multicellular organisms do so *via* changes in cell

numbers and cell sizes whereas unicellular organisms are constrained by a fixed ratio of adult: progeny cell size.

One size does not fit all

Although the usefulness of small organisms has been demonstrated, to inform ecological theory *via* micro- and mesocosm experiments and in natural systems (e.g. Petchey *et al.* 1999; Baulch *et al.* 2005; Reiss *et al.* 2010 and references therein), they are not simply small proxies of larger organisms and care should be taken when extrapolating observed patterns from microcosms to large scale, natural systems. Given their great abundance and diversity, they may have responses that are unique to them; indicated in this experiment by the lack of a power law function in the size spectra and the multi-modal body-size distribution plots.

Warming did not result in a change in the allometric slope of the species averaged size spectra which contrasts with previous work carried out in the ponds that showed the phytoplankton community show strong shifts in the size spectra and metabolic balance as a result of warming (Yvon-Durocher *et al.* 2010 a,b; Dossena *et al.* 2012). This may also be due to a sampling effect; as this study excluded prokaryotic organsisms e.g. cyanobacteria which have been shown to be important components of microbial communities, accounting for large proportions of primary production in marine systems (Sarmento *et al.* 2010).

In many studies of community size spectra, larger organisms are more susceptible to anthropogenic disturbances (e.g habitat changes such as warming) because they tend to have lower initial population densities and

greater energetic demands than smaller organisms (Kleiber 1947; Woodward *et al.* 2005). At the whole community level (disregarding functional groups), in this study, there is no significant effect of warming (Figure 3.1), possibly due to the multiple energy pathways that exist within the microbial loop (e.g. both living and non-living resources are used) which may enhance the stability of the community as a whole and dampen responses to habitat changes such as warming. In addition to this, size-dependent (e.g. predation) and size-independent (e.g. decomposition, bacterial mat grazing) interactions co-occur in microbial communities, so that the energy available to the individuals belonging to a particular size is not solely derived from smaller individuals in the food web. This may result in a breakdown of the classical body mass–trophic level relationships that form the basis of many studies of plankton communities that do not include the organisms examined in this study (e.g. Gaedke 1993; O'Connor *et al.* 2009; Winder *et al.* 2009; Yvon-Durocher 2010).

Caveats and future directions

The question remains as to whether this change in body size is due to intraspecific TSR effects or interspecific community shifts. Whilst this is not possible to determine precisely through this study, I suggest that the use of this system and more precise methodology (e.g use of coulter counters to measure body size of prokaryotes, to include in analyses of microbial size spectra), as suggested by Petchey and Belgrano (2010). This raises the question of distinction between the phenotypic change needed to cope with changes over a time scale shorter than the organism's life span and genotypic (evolutionary) change which takes place over much longer periods of time, thus there appears

to be no distinguishable “end-point” equilibria as a result of long-term warming in this experiment.

Increasingly, next generation sequencing methods are being used to enumerate and characterize the smallest organisms in a wide range of systems (Novias *et al.* 2011; Pilloni *et al.* 2012) from microcosm experiments (Bartram *et al.* 2011) to the arctic tundra (dos Santos *et al.* 2011). In future studies regarding microbial ecology and in testingsuch theories as the temperature size rule and the metabolic theory ofecology, the employment of sequencing techniques may help to define mechanisms behind such phenomena as reduced body size in response to warmingand changes in allometric scaling exponents which may, in future be linked to evolutionary patterns and gene expression (Finkel *et al.* 2005; Litchman *et al.* 2009).

3.6 References

Ashton, K.G. (2002) Patterns of within-species body size variation of birds: strong evidence for Bergmann's rule. *Global Ecology and Biogeography* **11**, 505-523

Ashton, K.G., Tracy, M.C., and Queiroz, A.D.(2010) Is Bergmann’s Rule valid for mammals? *The American Naturalist* **156**, 390-415

Atkinson, D. (1994) Temperature and organism size—A biological law for ectotherms *Adv. Ecol. Res.* **25**, 158

Atkinson, D. (1995) Effects of temperature on the size of aquatic ectotherms: Exceptions to the general rule. *Journal of Thermal Biology* **20**, 61-74

Atkinson, D., Ciotti, B. J., and Montagnes, D. J. S. (2003) Protists decrease in size linearly with temperature: ca. 2.5% °C–1. *Proceedings of the Royal Society of London. Series B: Biological Sciences* **270**, 2605-2611

Azam, F., Fenchel, T., Field J.G., Gray, J.S., Meyerreil, L.A.and Thingstad, F. (1983) The ecological role of water column microbes in the sea *Marine Ecology-Progress Series*, **10,** 257-263

Baulch, H. M., Schindler, D. W. and Turner, M. A. (2005) Effects of warming on benthic communities in a boreal lake: implications of climate change*Limnol. Oceanogr.* **50**, 437–452

Bartram A.K., Lynch M.D.J., Stearns J.C., Moreno-Hagelsieb G., Neufeld J.D. (2011) Generation of multimillion-sequence 16S rRNA gene libraries from complex microbial communities by assembling paired-end Illumina reads *Appl.Environ. Microbiol.* **77**: 3846–3852

Belgrano, A., Allen, A. P., Enquist, B. J. and Gillooly, J. F. (2002) Allometric scaling of maximum population density: a common rule for marine phytoplankton and terrestrial plants *Ecol. Lett.* **5**, 611–613

Berger, S.A., Diehl, S., Stibor, H., Trommer, G., Ruhenstroth, M., Jäger, C., Striebel, M. (2007) Water temperature and mixing depth affect timing and intensity of events during spring succession of the plankton *Oecologia* **150**, 643–654

Beveridge, O.S., Humphries, S., and Petchey, O.L. (2010) The interacting effects of temperature and food chain length on trophic abundance and ecosystem function *J. Anim. Ecol.* **79**, 693–700

Blanchard, J., Jennings, S., Law, R., Castle, M. D., McCloghrie, P., Rochet, M. and Benoit, E. (2009) How does abundance scale with body size in coupled size-structured food webs? *J. Anim. Ecol.* **78**, 270 – 280

Brown, J.H., Gillooly, J.F., Allen, A.P., Savage, V.M.and West, G.B. (2004) Toward a metabolic theory of Ecology *Ecology* **85,** 1771-1789

Carpenter, S.R. (1996) Microcosm experiments have limited relevance for community and ecosystem ecology *Ecology* **77**, 677–680

Cavender-Bares, K.K., Rinaldo, A., and Chisholm, S.W. (2001) Microbial size spectra from natural and nutrient enriched ecosystems *Limnol. Oceanogr.* **46**, 778–789

Chen, I.-C., Hill, J.K., Ohlemüller, R., Roy, D.B., and Thomas, C.D. (2011) Rapid range shifts of species associated with high levels of climate warming *Science* **333**, 1024

Damuth, J. (1981) Population-density and body size in mammals. *Nature* **290**, 699-700

Damuth, J. (1987) Interspecific allometry of population density in mammals and other animals: The independence of body mass and population energy-use *Biol. J. Linn. Soc.* **31**, 193–246

Daufresne, M., Lengfellner, K. and Sommer, U. (2009) Global Warming benefits the Small in Aquatic Ecosystems *PNAS,* **106**:31, 12788-12793

Dinmore, T.A. and Jennings, S. (2004) Predicting abundance-body mass relationships in benthic infaunal communities*Marine Ecology Progress Series* **276**:289–292

dos Santos, H.F., Cury, J.C., do Carmo, F.L., dos Santos, A.L., Tiedje, J., *et al.* (2011) Mangrove bacterial diversity and the impact of oil contamination revealed by pyrosequencing: bacterial proxies for oil pollution. *PLoS ONE* **6**: e16943

Dossena, M., Yvon-Durocher, G., Grey, J., Montoya, J.M., Perkins, D.M., Trimmer, M., and Woodward, G. (2012) Warming alters community size structure and ecosystem functioning *Proc. R. Soc. B.* **279**, 3011-3019

Ducklow, H.W., Purdie, D.A., Williams, P.J. leB. and Davies, J.M. (1986) Bacterioplankton: A sink for carbon in a coastal marine plankton community *Science* **232,** 865–867

Elser, J.J. and Urabe, J. (1999) The stoichiometry of consumer-driven nutrient recycling: theory, observations and consequences*Ecology*, **80**, 735–751

Finkel, Z. V., Katz, M. E., Wright, J. D., Schofield, O.M.E. and Falkowski, P.G. (2005) Climatically driven macroevolutionary patterns in the size of marine diatoms over the Cenozoic*Proc. Natl Acad. Sci. USA,* **102**, 8927–8932

Forster, J., Hirst, A.G. and D. Atkinson, D. (2011a) How do organisms change size with changing temperature? The importance of reproductive method and ontogenetic timing *Functional Ecology* **25:** 1024–1031

Forster, J., Hirst, A. G. and Woodward, G. (2011b) Growth and development rates have different thermal responses *Am. Nat.* **178**, 668–678.

Gaedke, U. (1993) Ecosystem analysis based on biomass size distributions: a case study of a plankton community in a large lake *Limnol. Oceanogr.* **38**, 112 – 127

Gillooly, J. F., Brown, J. H., West, G. B. and Savage, V. M. (2001) Effect of size and temperature on metabolic rate *Science*, **239**, 2248 – 2251

Gillooly, J. F., Charnov, E. L., West, G. B., Savage, V. M. and Brown, J.H. (2002) Effects of size and temperature on developmental time *Nature*, **417**,70 – 73

Gasol, J.M., Guerrero, R., and Pedrosalio, C. (1991) Seasonal variations in sizestructure and prokaryotic dominance in sulphurous Lake Ciso. *Limnol. Oceanogr.* **36**, 860–872

Griffiths, D. (1998) Sampling effort, regression method, and the shape and slope of size-abundance relations. *J. Anim. Ecol.* **67**, 795–804

Hillebrand, H., Dürselen, C.-D., Kirschtel, D., Pollingher, U., and Zohary, T. (1999) Biovolume calculation for pelagic and benthic microalgae *Journal of Phycology* **35**, 403-424

IPCC (2007) in Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*Ed. Parry, M.L., Canziani, O.F., Palutikof, J.P., van der Linden, P.J. and Hanson, C.E. (Cambridge University Press, Cambridge) pp. 7-22*

Jacobsen, D., Schultz, R. and Encalada, A. (1997) Structure and diversity of stream invertebrate assemblages: the influence of temperature with altitude and latitude *Freshwater Biology*, **38**: 247–261

James, F. C. (1970) Geographic Size Variation in Birds and Its Relationship to Climate *Ecology* **51**, 365-390

Jennings, S. and Mackinson, S. (2003) Abundance–body mass relationships in size-structured food webs *Ecol. Lett.* **6**, 971–974

Kerr, S.R. and Dickie, L.M. (2001) The Biomass Spectrum: A Predator Prey Theory of Aquatic Production,*Columbia University Press, New York*

Kleiber, M. (1947) Body size and metabolic rate *Physiological Reviews* **27**, 511–541

Lindeman, R.L. (1942) The Trophic Dynamic Aspect of Ecology *Ecology*, 23(4), 399-417

Litchman, E., Klausmeier, C.A. and Yoshiyama, K. (2009) Contrasting size evolution in marine and freshwater diatoms *Proc. Natl. Acad. Sci.USA* **106**, 2665 – 2670

Melian, C. J., Vilas, C., Baldo, F., Gonzalez-Ortegon, E., Drake, P., and Williams, R. J. (2011) Eco-evolutionary dynamics of individual-based food webs Pages 225-268 *inA. R. J. Belgrano, editor. Advances in Ecological Research, Vol 45: The Role of Body Size in Multispecies Systems*

Miller, K.G., Wright, J.D. and Browning, J.V., (2005) Visions of ice sheets in a greenhouse world*Marine Geology* **217**, 215-231

Moss, B., McKee, D. and Atkinson, D. (2003) How important is climate? Effects of warming, nutrient addition and fish on phytoplankton in shallow lake microcosms*J. Appl.Ecol.* **40**, 782–792

Newsham, K.K., and Garstecki, T. (2007) Interactive effects of warming and species loss on model Antarctic microbial food webs. *Funct. Ecol.* **21**, 577–584

Novais, R.C., Thorstenson, Y.R., (2011) The evolution of Pyrosequencing (R) for microbiology: From genes to genomes*J. Microbiol. Meth.* **86**: 1–7

O'Connor, M.I., Piehler M.F., Leech, D.M., Anton, A., Bruno, J.F. (2009) Warming and resource availability shift food web structure and metabolism*PLoS Biol* **7(8)**: e1000178. doi:10.1371/journal.pbio.1000178

O'Gorman, E.J., Pichler, D.E., Adams, G., Benstead, J.P., Craig, N., Cross, W.F., Demars, B.O.L., Friberg, N. Gísli Mar Gíslason, Rakel Gudmundsdóttir, R.,

Hawczak, A., Hood, J.M., Hudson, L.N., Liselotte Johansson, L., Johansson, M., Junker, J.R., Laurila, A., Manson, J.R., Mavromati, E., Nelson, D., Ólafsson, J.S., Perkins, D.M., Petchey, O.L., Plebani, M., Reuman, D.C., Rall, B.C., Stewart, R., Thompson, M.S.A. and Woodward, G. (2012) Impacts of warming on the structure and function of aquatic communities: individual- to ecosystem-level responses *An. Ecol. Rev.* **47,** 81-176

Parmesan, C. and Yohe, G. (2003) globally coherent fingerprint of climate change impacts across natural systems *Nature* **421**, 37-42

Parmesan, C. (2006) Ecological and evolutionary responses to recent climate change*Annual Review of Ecology Evolution and Systematics* **37,** 637-669

Pascual, M.M. and Dunne, J.A. (2005) Ecological networks: linking structure to dynamics *Oxford, UK: Oxford University Press*

Petchey, O., Mcphearson, P., Casey, T.and Morin, P. (1999) Environmental warming alters food-web structure and ecosystem function *Nature* **402,** 69-72

Petchey, O. L., Beckerman, A. P., Riede, J. O., and Warren, P. H. (2008) Size, foraging, and food web structure *Proceedings of the National Academy of Sciences of the United States of America* **105**, 4191-4196

Petchey, O.L. and Belgrano, A. (2010) Body-size distributions and size-spectra: universal indicators of ecological status? *Biol. Lett.* doi:10.1098/rsbl.2010.0240

Peters, R.H. (1983) The ecological implications of body size Cambridge University Press, Cambridge

Pilloni, G., Granitsiotis, M.S., Engel, M., Lueders, T. (2012) Testing the Limits of 454 Pyrotag Sequencing: Reproducibility, Quantitative Assessment and Comparison to T-RFLP Fingerprinting of Aquifer Microbes*PLoS ONE* **7**,7:e40467. doi:10.1371/journal.pone.0040467

R Development Core Team (2011) R: A language and environment for statistical computing*R Foundation for Statistical Computing, Vienna, Austria ISBN 3-900051-07-0*, URL http://www.R-project.org/

Reiss, J. and Schmid-Araya, J. M. (2008) Existing in plenty: abundance, biomass and diversity of ciliates and meiofauna in small streams. *Freshwater Biology* **53**, 652 – 668

Reiss, J., Bridle, J.R., Montoya, J.M. and Woodward, G. (2009) Emerging horizons in biodiversity and ecosystem functioning research*Trends Ecol. Evol.* **24**, 505–514

Reiss, J., Forster, J., Cassio, F., Pascoal, C., Stewart, R., Hirst, A.G. (2010) When Microscopic Organisms Inform General Ecological Theory Ed: Woodward, G.*Integrative Ecology: From Molecules to Ecosystems Book Series: Advances in Ecological Research* **43,** p. 45-85

Sarmento, H., Montoya, J.M., Vazquez-Dominguez, E., Vaque, D. and Gasol, J.M. (2010) Warming effects on marine microbial food web processes: how far can we go when it comes to predictions? *Phil. Trans. R. Soc. B.* **365**, 2137 – 2149

Schmid, P.E., Tokeshi, M. and Schmid-Araya, J.M. (2000) Relation between population density and body size in stream communities*Science* 289, 1557–1560

Schmid, P.E., Tokeshi, M., and Schmid-Araya, J.M. (2002) Scaling in stream communities *Proc. R. Soc. Lond. B Biol.* **269**, 2587–2594

Sheldon, R. W., Prakash, A. & Sutcliffe, W. H. J. (1972) The size distribution of particles in the ocean *Limnol. Oceanogr.***17,** 327–340. (doi:10.4319/lo.1972.17.3.0327)

Sheridan, J.A. and Bickford, D. (2011) Shrinking body size as an ecological response to climate change *Nature Climate Change* **1**, 401-406

Shin, Y.J., Rochet, M.J., Jennings, S., Field, J.G. and Gislason, H. (2005) Using size-based indicators to evaluate the ecosystem effects of fishing. *ICES J. Mar. Sci.* **62**, 384–396

Stead, T.K., Schmid-Araya J.M. and Hildrew, A.G. (2003) All creatures great and small: patterns in the stream benthos across a wide range of metazoan body size *Freshwater Biology* **48,** 532–547

Steffen, W., Crutzen, P. J., and McNeill, J. R. (2007) The Anthropocene: are humans now overwhelming the great forces of nature*AMBIO: A Journal of the Human Environment* **36**, 614-621

Walters, R.J., Hassall, M., (2006) The temperature-size rule in ectotherms: may a general explanation exist after all? *Am. Nat.* **167**: 510–523

Walther, G. R. (2010) Community and ecosystem responses to recent climate change *Phil. Trans. R. Soc. B.* **365**, 2019–2024

West, G.B., Brown, J.H., Enquist, B.J., (1997) A general model for the origin of allometric scaling laws in biology*Science,* **276**, 122-126

Winder, M. and Schindler D.E., (2004) Climate change uncouples trophic interactions in an aquatic ecosystem *Ecology* **85**, 2100– 2106

Winder, M., Reuter, J.E. and Schladow, S.G. (2009) Lake warming favours small-sized planktonic diatom species *Proc. R. Soc. B.* **276**, 427-435

White, E.P., Ernest, S.K.M., Kerkhoff, A.J. and Enquist, B.J. (2007) Relationships between body mass and abundance in ecology *Trends. Ecol. Evol.* **22**, 323–

White, E.P., Enquist, B.J. and Green, J. (2008) On estimating the exponent of power-law frequency distributions *Ecology*, **4**, 905–912

Woodward, G., Speirs, D.C. and Hildrew, A.G. (2005) Quantification and resolution of a complex, size- structured food web *Adv. Ecol. Res.* **36**, 85 – 135

Woodward, G., Perkins, D. M. and Brown, L. E. (2010) Climate change and freshwater ecosystems: impacts across multiple levels of organization*Phil. Trans. R. Soc. B* **365**, 2093–2106

Yvon-Durocher, G., Montoya, J. M., Trimmer, M. and Woodward, G. (2010) Warming alters the size spectrum and shifts the distribution of biomass in freshwater ecosystems*Global Change Biology* **17**, 1681–1694

Yvon-Durocher, G., Reiss, J., Blanchard, J., Ebenman, B., Perkins, D.M., Reuman, D. C., Thierry, A., Woodward, G. and Petchey, O. (2011) Across ecosystem comparisons of size structure: methods, approaches and prospects *Oikos*, **120**, 550 – 563

Chapter 4

Community composition of microbial-meiofaunal assemblages in experimentally warmed mesocosms

4.1 Abstract

With rising global temperatures, there are pressing questions regarding the future of species which humans rely on for the maintenance of ecosystem function. The organisms of the microbial loop provide a critical link from basal to higher trophic levels, by nutrient cycling, carbon assimilation and the essential processes behind these functions. The microbial loop as a whole has been overlooked in climate change studies to date, especially in terms of addressing common responses to global warming (e.g. phenological shifts). I attempt to address this and make use of an ongoing global warming, mesocosm experiment to investigate the effect of a temperature rise of ~3-5 °C on the abundance and biomass of five major microbial loop taxa (algae, flagellates, ciliates and meiofauna). I asked the following questions: (i) How does warming affect the abundance and biomass of the 4 taxa focused upon? (ii) Does warming alter the seasonal and spatial dynamics in terms of abundance and biomass within each taxonomic group (i.e. do they exhibit phenological changes, as is a common response to climate change? (iii) Does warming influence the body size of individuals within major taxonomic groups – as a reduction in the body size of ectotherms is a widely observed response to

warming and finally, (iv) how might the responses to warming in the microbial loop ramify through to higher levels of organisation given the varied roles that small organisms play in natural systems?

Results showed that (i) treatment alone had no overall significant effect on the abundance and biomass of the 4 taxa examined across the sampling period, (ii) both abundance and biomass was influenced by warming indirectly, shown by a significant interaction of treatment with sampling occasion and position in the water column and (iii) individuals tended to have smaller body masses, on average, in warmed mesocosms. Given the wide range of roles the small organisms play in natural systems, the implications of phenological shifts as a result of warming are far-reaching in terms of species interactions and effects at the higher levels (e.g. food web).

4.2 Introduction

Global warming: impacts on communities

Water temperature and availability are climate dependent and one of the predictions made by the IPCC (2007) is that global surface temperatures will rise by between 3-5 °C under scenario A1FI during the next century, as a direct result of anthropogenic activity and the rapid changes this has brought about e.g. elevated levels of greenhouse gases like CO_2 (Miller *et al.* 2005; Steffen *et al.* 2007). This predicted future warming has important consequences at all levels of biological organisation, from individuals, to entire ecosystems (Walther *et al.* 2002; Montoya and Raffaelli 2010; Walther 2010; Woodward et al. 2010a,b, O'Gorman *et al.* 2012). The impact of this temperature rise on aquatic systems is important because humans depend on ecological systems for

resources and ecosystem services (e.g. nutrient cycling and carbon sequestration). Temperature sets the pace of life and therefore, is one of the main abiotic factors influencing the physiological performance of individual ectothermic organisms (which drive the functions and services humans depend on), it is important that potential impacts of warming on individuals, populations and community assemblages is fully understood. Many studies have addressed how climate change impacts individuals, populations and communities in a wide range of environments across the globe (e.g. Walther *et al.* 2002; Root *et al.* 2004; Parmesan and Yohe 2003; Parmesan 2006 and references therein;) and fewer address the impact of warming at higher levels of organisation due to difficulties in linking structure to function (but see Petchey *et al.* 1999; Walther *et al.* 2002; Montoya and Raffaelli 2010; Dossena *et al.* 2012; O'Gorman *et al.* 2012).

Organisms of the microbial loop occupy a critical niche in food webs as predators of bacteria and fungi (Ekelund and Rønn 1994; Foissner 1987), as food sources for multicellular microscopic animals such as nematodes, which in turn may act as a food source for higher taxa [e.g. fish (Small 1987; Alheitand Scheibel 1982)]. In addition, they play a key role in nutrient cycling and trophic flux to higher levels of organisation (e.g Hillebrand and Matthiessen 2009; Hooper *et al.*, 2005; Pierce and Turner 1992; Reiss *et al.*, 2009). These organisms are taxonomically diverse (Fenchel 1987; Finlay and Esteban 1998), ecologically diverse (Finlay and Esteban 1993,1997,1998; Patterson 1999) as well as extremely abundant (Finlay *et al.* 1988, 1996; Reiss and Schmid-Araya 2008) and it is now widely recognised that they are functionally important in aquatic environments (Fenchel 1975; Finlay *et al.* 1997; Reiss *et al.* 2008;

Schmid *et al.* 2000; Stead *et al.* 2003, 2005; Reiss and Schmid-Araya 2008; Williams 1981; Reiss *et al.* 2010) yet it remains unclear how unprecedented rates of warming will alter the structure and by inference, the function of the microbial loop; that is to say, the connection between the traits of individuals, the structure of microbial communities and ultimately, ecosystem function is not explicit and a longstanding division between community and ecosystem ecology has hindered this linkage (Purdy *et al.* 2010). Which traits are important, or how they interact with one another or respond to environmental change, is still poorly defined even for plants and animals (Reiss *et al.* 2009) and often entirely undefined for the microbial components of an ecosystem. Nevertheless, attempts have been made using microcosms and mesocosms to link community structure to ecosystem functioning; Petchey *et al.* (1999) linked structure to function in microcosm food webs and recently, attempts to address this research gap and relate benthic community structure to ecosystem function has been carried out using the same mesocosm experiment as this study (Yvon-Durocher *et al.* 2010 a, b; Dossena *et al.* 2012). These previous studies highlighted the effects of warming on the body size and abundance distribution of phytoplankton (Yvon-Durocher 2010a) and benthic macroinvertebrate community structure to rates of decomposition (Dossena *et al.* 2012). Most climate change studies to date, in natural systems, have often been confounded by latitudinal and altitudinal gradients (e.g. Jacobsen *et al.* 1997) and surveys are correlational at best whereas microcosm and mesocosm experiments can detect causal relationships (e.g. between temperature and abundance). In this experiment, the mesocosms act as replicated ecosystems, excluding

confounding effects of latitude and altitude but do sacrifice realism that can only come from studies in natural systems (Carpenter *et al.* 1996).

Recently, the biological consequences of elevated temperatures have been re-evaluated to determine potential shifts in body size as well as phenology, geographic distribution, species diversity and primary productivity (Daufresne *et al.* 2009; Hill *et al.* 1999; Richardson and Schoeman 2005; Ruess *et al.* 1999; Weitere *et al.* 2009). Thermal tolerances as well as the capability of thermal adaptation are essential components within the microbial loop because growth and division rates are directly dependent on temperature.

Body size is a key determinant of community structure and its relationship with abundance can describe how biomass is partitioned among the biota (Elton 1927; Lindeman 1942; Damuth 1981; Peters 1983; Brown *et al.* 2004; Petchey *et al.* 2008). This size structure influences ecological processes across multiple levels of organisation, from individuals (Peters 1983; Brown *et al.* 2004), their interactions (Emmerson and Raffaelli 2004; Berlow *et al.* 2009) to populations (Damuth 1981; Jennings and Mackinson 2003; Reuman *et al.* 2008), communities and ultimately, ecosystems (Petchey *et al.* 2008). Previous work on the impact of warming on community and ecosystem properties, using the same mesocosm carried out by Yvon Durocher *et al.* (2010 a,b) who showed that warming is responsible for a shift in the phytoplankton size spectra, towards smaller body sizes (and *chapter 3* of this thesis) and that warming alters the metabolic balance of the whole ecosystem by elevated respiration compared to photosynthesis in warmed ponds. Both of these studies have shown shifts in the body size distribution among the phytoplankton but not in the zooplankton; this indicates that photosynthetic organisms may be more

susceptible to warming effects and hints at a possible shift towards heterotrophy over autotrophy as a result of warming. This apparent shift may be reflected in the abundance and biomass of microbial loop taxa and determined byexamining the community structure of broad taxonomic groups.

Understanding both taxonomic and functional changes within populations or groups (e.g. shifts in the abundance and body size of heterotrophs and autotrophs) and how this might induce changes at the ecosystem level will aid future management of freshwater systems as a whole and facilitate more accurate predictions regarding the consequences of elevated rates of warming (or other aspects of anthropogenic climate change) on freshwater systems. The microbial loop organisms are a particularly useful and interesting group of organisms in this context because of their functional and taxonomic diversity in natural systems (Finlay and Esteban 1998). It is therefore essential, to aid future predictions of how ecosystems function, that we understand how even the smallest organisms, might be impacted by anthropogenic stressors such as rising global temperatures.

In the *chapter 3* of this thesis, I addressed individual based size spectra, disregarding taxonomy and focused instead on the whole community size spectra of coarsely defined functional groups (autotrophs and heterotrophs only). Autotrophic protists (e.g. desmids and diatoms) are responsible for the bulk of primary production in most aquatic habitats (Reiss *et al.* 2010 and references therein) and have high secondary production rates, operating at the base of food webs. The distinction between autotrophic individuals and heterotrophic individuals present throughout the sampling period provided insights into whether warming promotes a shift towards heterotrophy in the

microbial loop of these ponds and separate potential effects on primary (autotrophic) and secondary (heterotrophic) production [see Yvon-Durocher *et al.* (2010a) and *chapter 1* of this thesis]. To develop this further, in terms of the microbial taxa present in the ponds, this chapter addresses how warming will impact community composition with a broad focus on 4 major taxonomic groups of the microbial loop (algae, flagellates, ciliates, meiofauna) from three depths in shallow ponds and spanning 12 months. The seasonal patterns of these small organisms have been examined in past studies (Smetacek 1981; Carrick and Fahenstiel 1990; Schmid-Araya 1994,1997) but introducing the spatial and temporal gradient allowed examination of the potential for more subtle effects of temperature on the phenology of these small organisms which has been studied very little in the past (but see Carrick et al. 1991; Stead *et al.* 2003; Winder and Schlindler 2004).

In terms of phenology, this study focuses on the periodic peaks in biomass and abundance of the taxa described above and the effect that warming may have on the respective values for each. By sampling monthly, it is possible to discern firstly, the seasonal patterns for both warmed and ambient treatments and test for differences between the two. With reference to current theories regarding body size and warming, I hypothesise the following:

(i) Warming will have a significant, observable effect on the abundance and biomass of the taxa examined, sampled from the mesocosms. There will be increased abundance of these small taxa in warmed mesocosms, compared to ambient, reflecting current theory that warming favours smaller bodied organisms (Daufresne *et al.* 2009).

(ii) There will be a measurable shift in phenology, evident by a significant interaction term between treatment and sampling month for both abundance and biomass (see *chapter 1* of this thesis).

(iii) In addition to changes in seasonal patterns, warming will influence the spatial pattern of abundance within the major groups, evident by a significant interaction between treatment and sample depth.

4.3 Methods

Study Site

The experiment was part of an ongoing project set up in December 2006 at the Freshwater Biological Association River Laboratory, East Stoke, Dorset, UK. A detailed description of the experimental set-up is found in Yvon-Durocher *et al.* (2010 a,b) as well as in the study site chapter of this thesis. Briefly, it consisted of 20 freshwater mesocosms (~1m^3, 0.5m water depth): ten replicates were left at ambient temperature whilst the other 10 were warmed to 3.0-5.0 °C (mean 4 °C) above ambient and had previously been seeded with benthic and pelagic taxa from the nearby river Frome and allowed to establish for a year before warming commenced (see *chapter 2*).

Sampling

Samples of 10-ml were collected from each of the sediment, surface and mid-column of all 20 mesocosms, every month from February 2009 until March 2010 inclusive. Detailed sampling methods are found in the general methods chapter of this thesis. 1-ml sub samples were removed from each and placed in a Sedgwick rafter counting cell was used for counting and identification of live organisms, under light microscopy using an Olympus BX50 microscope at 40x400x magnification (Olympus Optical, Tokyo, Japan).

Identification and quantification

For the taxonomic assessment of the community composition of the microbial loop organisms present in the ponds, over the sampling period, organisms were identified live within a 1-ml Sedgwick-Rafter counting cell, using the following

keys: Kahl (1930, 1931, 1932, 1935); Bick (1972); Corliss (1979); Curds*et al.* (1982, 1983); Foissner *et al.* (1991,1992, 1994, 1995).

Body size measurements

Live individuals were photographed and individual body sizes were measured using Image analysis software (Image J, Q Capture) under light microscopy using a Nikon SMZ-U stereomicroscope. Individual body dimensions (length and width) were converted to specific biovolume using common geometric formulae (Hillebrand *et al.* 1999). The biovolume of the protists (ciliates and flagellates) was converted into carbon content assuming 0.14-pgC μg^3 (Putt and Stockner 1989; Reiss and Schmid Araya 2008). Meiofaunal biovolume was converted into individual body mass by assuming a specific gravity of 1.1 and individual carbon content was estimated assuming a dry/wet weight ratio of 0.25 and a dry carbon content of 40% (Feller and Warwick 1988; Reiss and Schmid-Araya 2008).

Data Analysis

Initially, principal component analysis (PCA) was performed, using CANOCO software (Microcomputer Power, Ithaca, NY, USA), on the whole microbial community for the whole sampling period. Absolute numerical abundance and biomass of all groups identified during the sampling period were compared using repeated measures ANOVAs (RMANOVAs) with the mesocosms fitted as random effects for each variable. The main effects included in the model were treatment, month (of sampling) and depth to investigate potential two-way and three-way interactions between treatment and sampling month (hypothesis (ii),

a treatment-season interaction) and between treatment and depth to (hypotheses (iii), a treatment-depth interaction regarding a possible spatial and seasonal context of global warming for small organisms.

4.4 Results

Community composition

PCA analysis revealed the expected, strong spatial and temporal pattern of abundance in the ponds (Figure 4.1, Table 4.1). There was no overall significant effect of warming *per se* at the community level for any of the taxa identified and measured from the mesocosms.

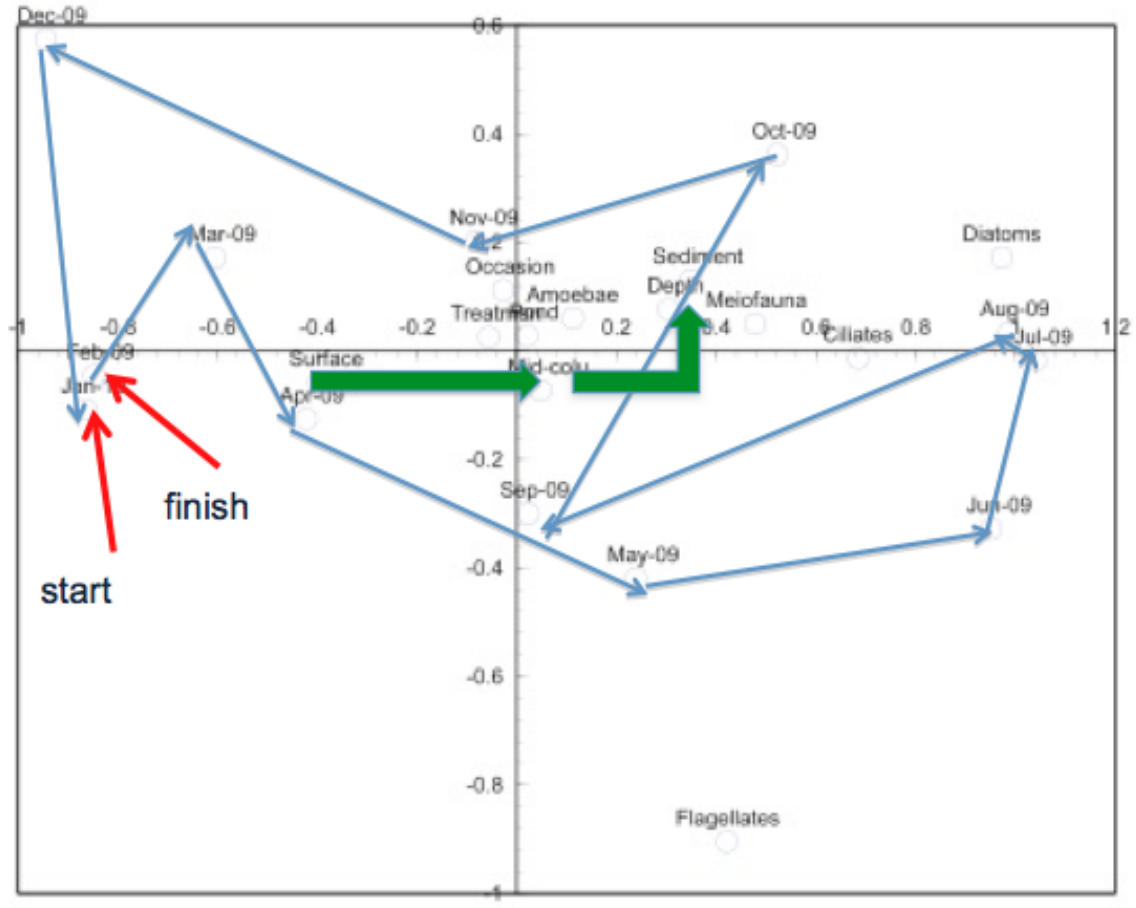


Figure 4.1 Principal components analysis of community composition in all ponds across the whole sampling period (February 2009-January 2010). Axis 1 of the principal component explains 37.3% of species variation and is likely to be a temporal effect. Axis 2 explains 33% of the species variation and there is a spatial effect – there are distinct differences in species depending on where they are in the water column. 'Start' and 'Finish' labelling indicates the commencement and cessation of sampling respectively. Blue arrows highlight the seasonal gradient and gree arrows highlight the spatial gradient.

Table 4.1 Significant predictors of relative abundance of all groups in ponds as found in a PCA.Eigenvalues and cumulative percentage variation of species data on the 1st and 2nd axis are given for the model including significant predictors. Predictors found to influence the relative abundance of taxa are shown with their F-ratios and P-values (*<0.05; **<0.01; ***< 0.001; NS, not significant. Pond refers to individual mesocosms and block refers to the experimental design (described in *chapter 2*).

	Groups in all mesocosms (PCA)
1st Axis (%)	37.3
2nd Axis (%)	33
1st+2nd Axis (%)	70.3
Month	7.65***
Depth	5.15**
Treatment	NS
Pond	NS
Block	NS

Effects of warming on abundance and biomass

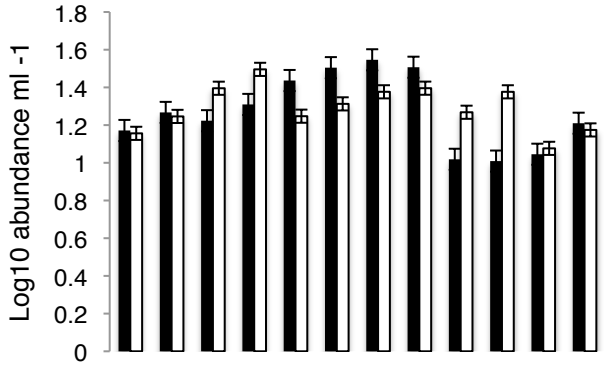
Hypothesis (i) was unsupported, as temperature did not have a significant overall effect on the abundance and biomass of 3 out of the 4 groups tested. Flagellates did show a significant response to warming in abundance ($F_{1,19}$=23.62, P=0.035) but not in biomass ($F_{1,19}$=3.21, P=0.08 (see Table 4.4, Figure 4.3 and Figure) and were the only group that exhibited a response to temperature in terms of abundance. All the groups examined exhibited significant responses to month, indicating strong seasonality in both abundance and biomass (see Tables 4.3-4.6 for taxon-specific RMANOVA results). The

abundance and biomass of the heterotrophic protists, which included ciliates, heterotrophic flagellates and amoebae, showed a significant response to a three-way interaction term between treatment, month and depth.

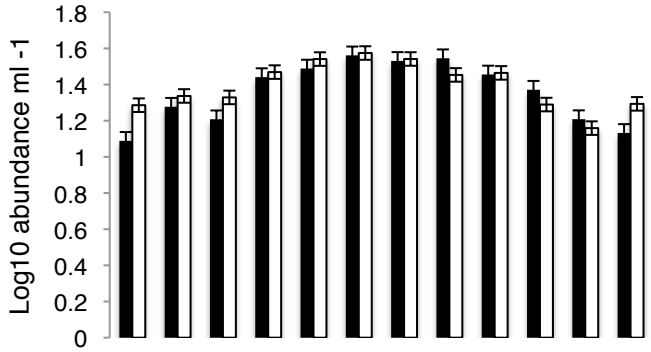
Abundance time series

ALGAE

SURFACE



MID COLUMN



SEDIMENT

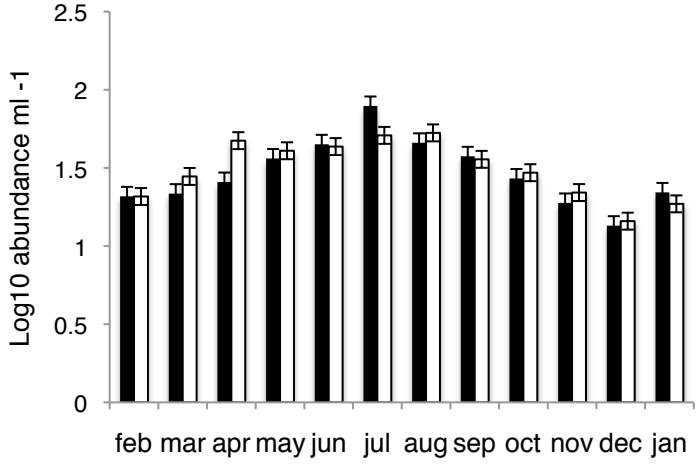


Figure 4.2 Mean Log_{10} abundance ml $^{-1}$(±1SE) of algae (desmids and diatoms only) across the sampling period February 2009-January 2010, in the sediment, mid-column and surface.

FLAGELLATES

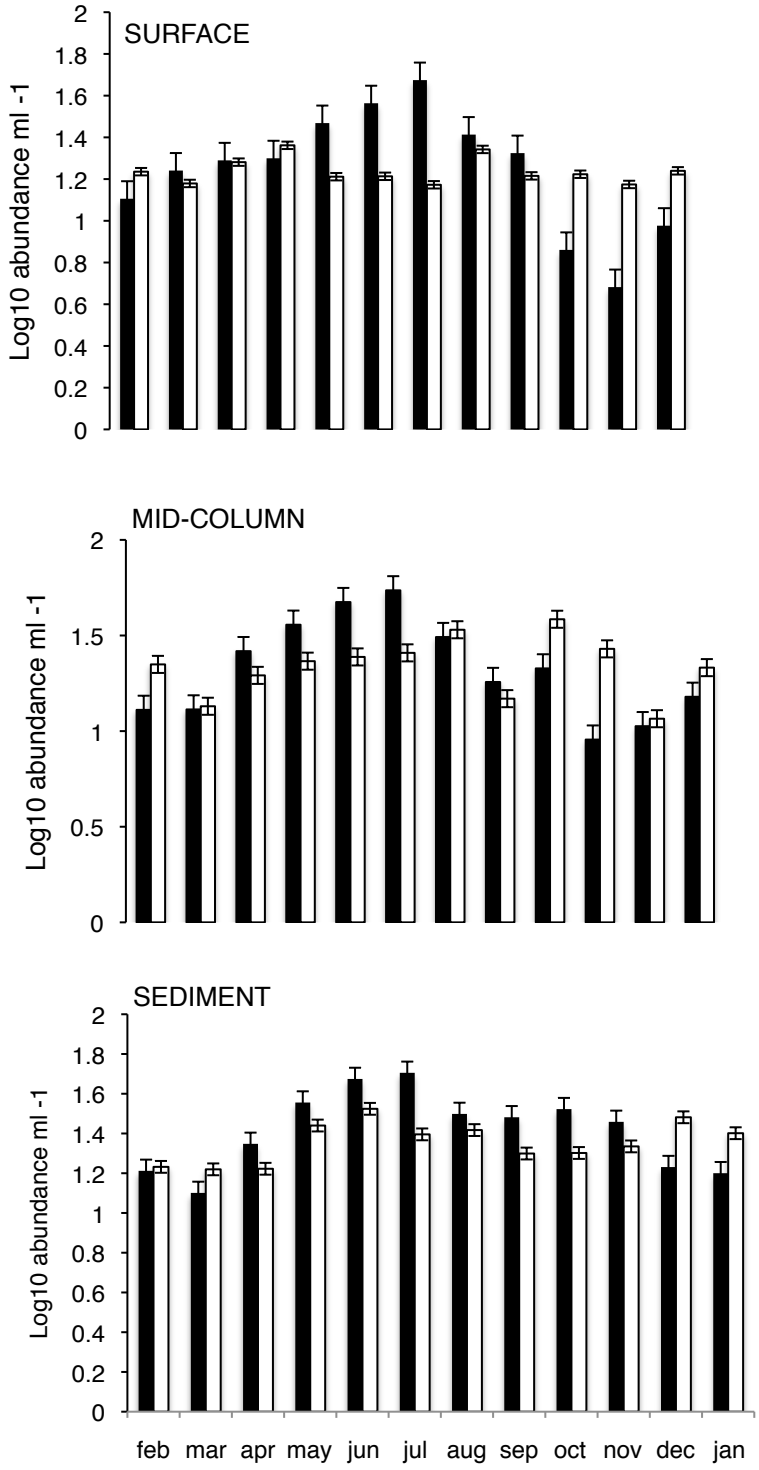


Figure 4.3 Log_{10} abundance ml^{-1} (±1SE) of autotrophic flagellates across the whole sampling period, for ambient (black bars) and warmed ponds (white bars). The effect of warming on flagellate abundance is most pronounced during the summer, in the surface of the ponds and during the winter, in the sediment (see Table 4.1)

HETEROTROPHIC PROTISTS

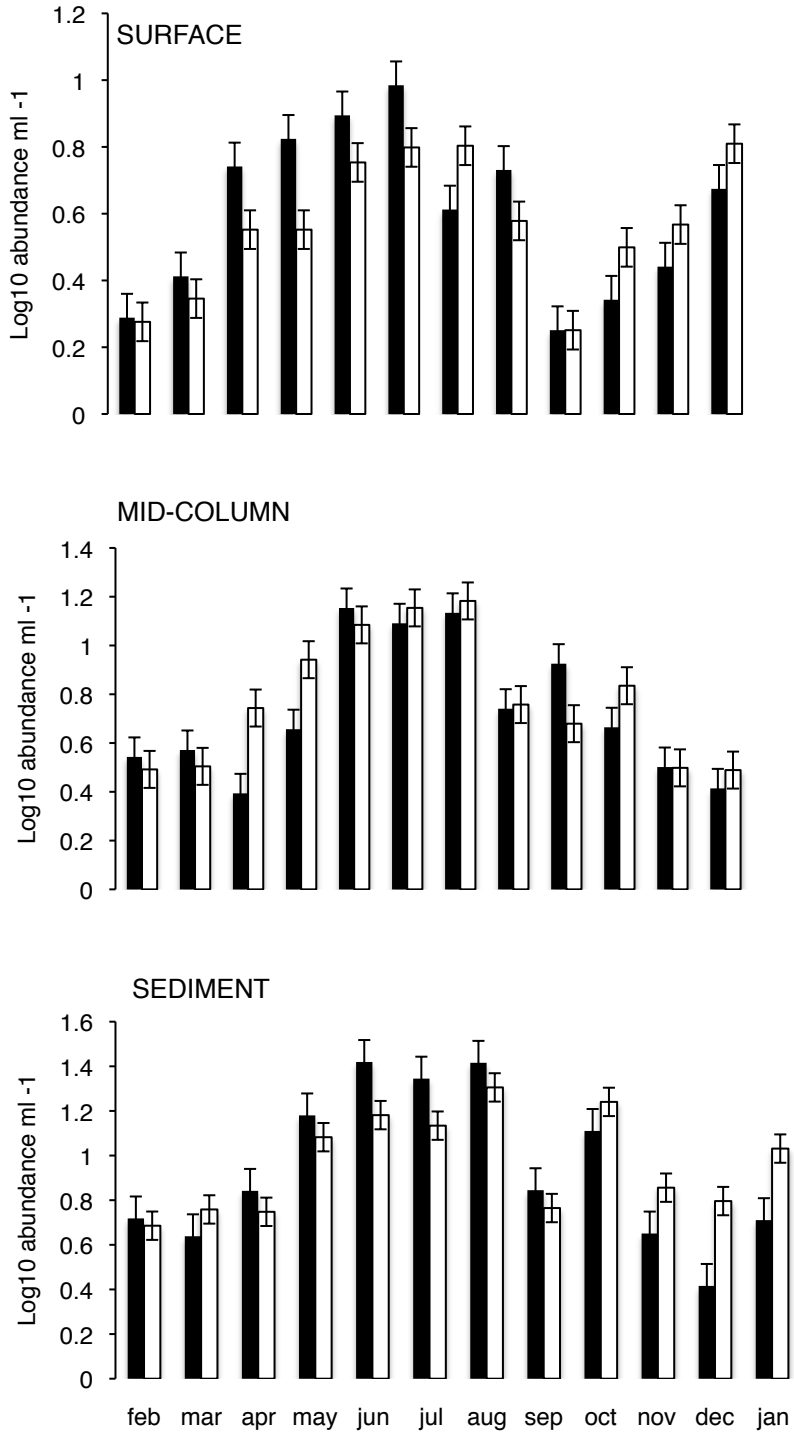


Figure 4.4 Log_{10} numerical abundance of heterotrophic protists in the mesocosms, at each depth; Surface, Mid-column and Sediment, by treatment (black bars are ambient ponds, white bars represent warmed ponds, across the whole sampling period from February 2009 until January 2010.

MEIOFAUNA

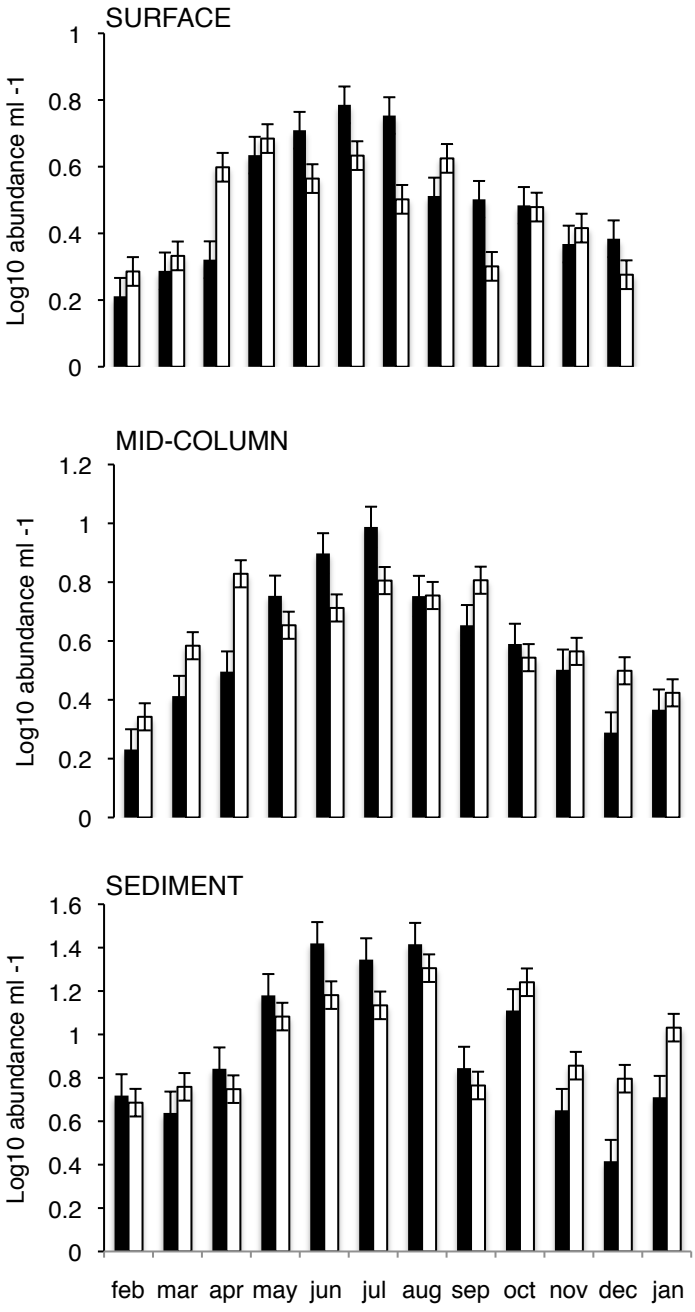


Figure 4.5 Log_{10} abundance of meiofauna (±1SE) across the sampling period February 2009-January 2010, at each depth and per treatment. The black bars represent ambient ponds, white bars represent warmed ponds.

Biomass time series plots

ALGAE

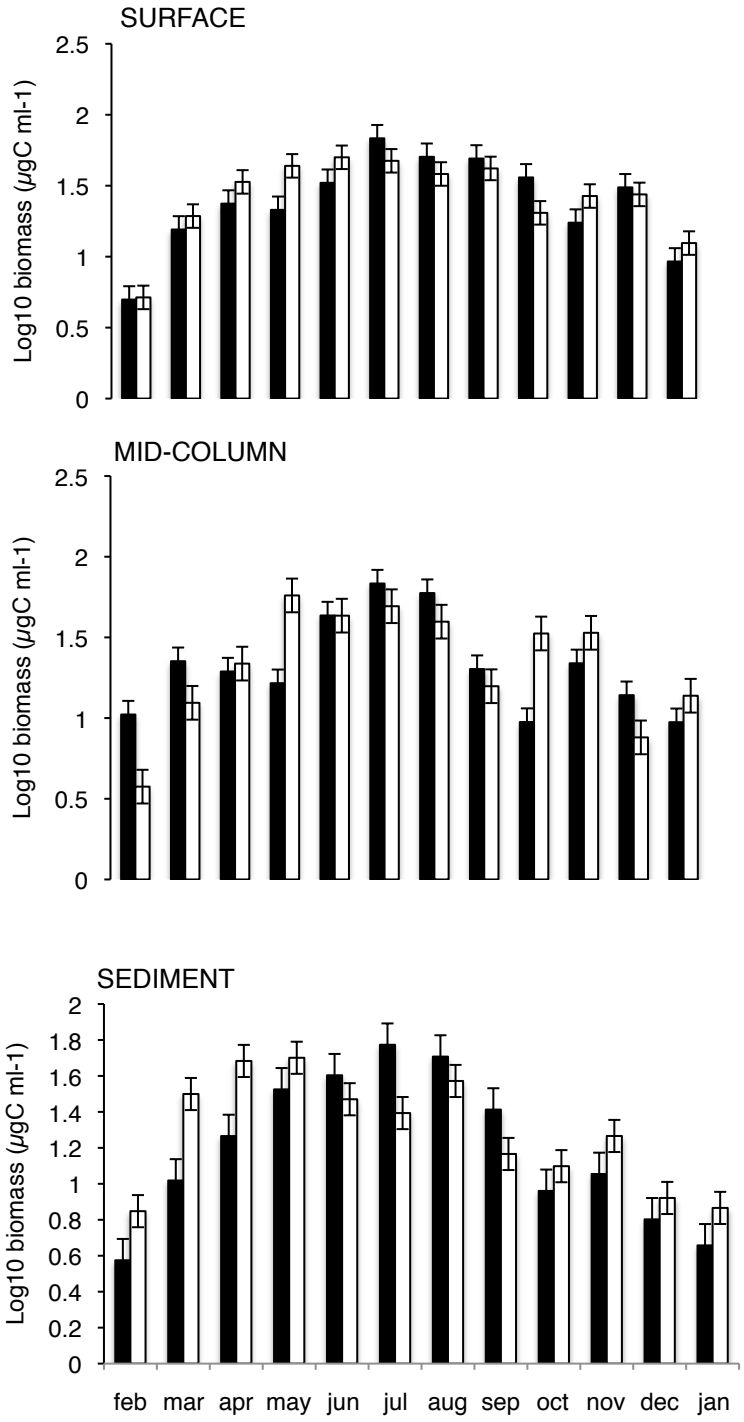


Figure 4.6 Log_{10} biomass (μgC ml^{-1}) (±1SE) for the algae in the ambient (black bars) and warmed (white bars) mesocosms, at each depth sampled; surface, mid-column and sediment.

FLAGELLATES

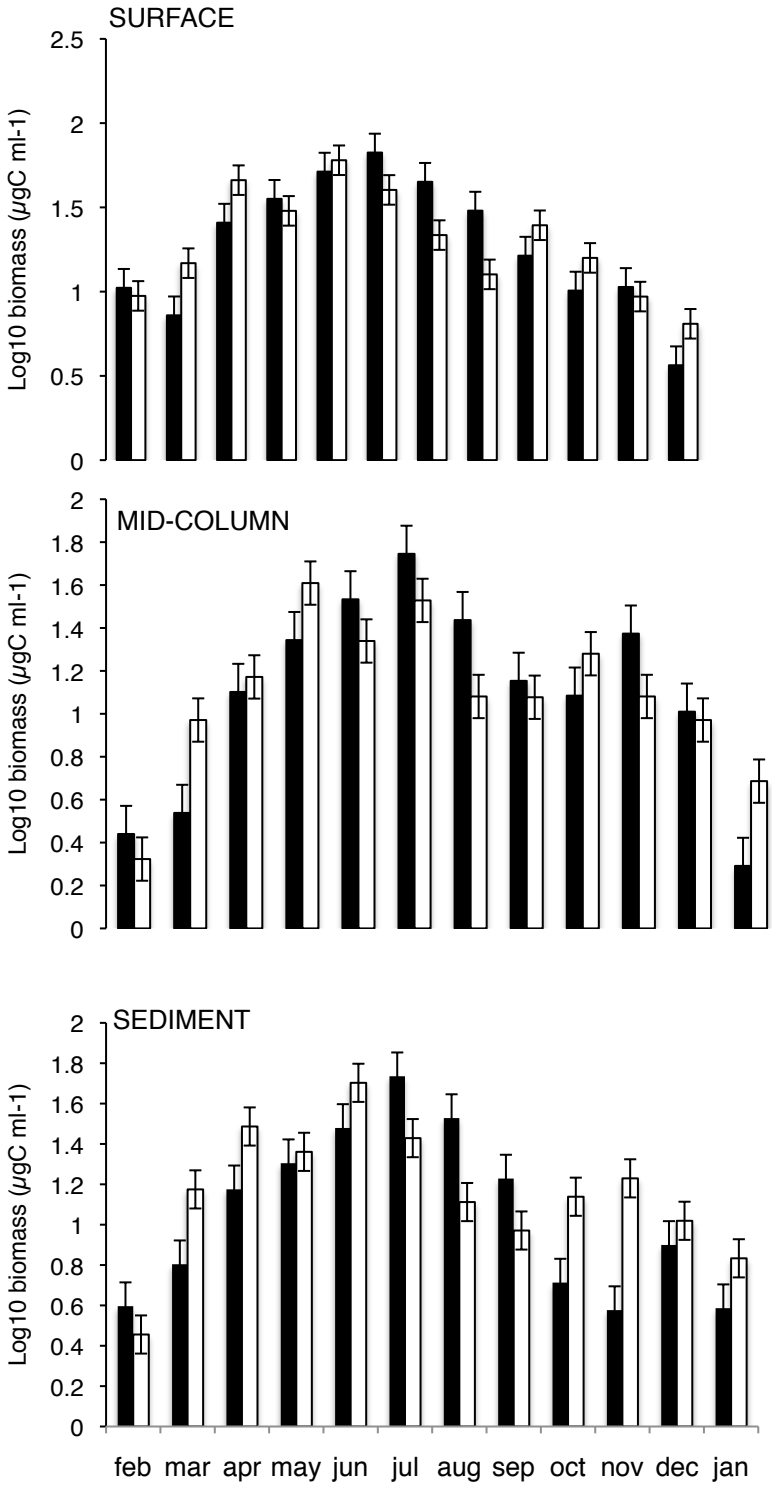


Figure 4.7 Log_{10} biomass (μgC ml^{-1}) (±1SE) for the algae in the ambient (black bars) and warmed (white bars) mesocosms, at each depth sampled; surface, mid-column and sediment, across the sampling period from February 2009 to January 2010

HETEROTROPHIC PROTISTS

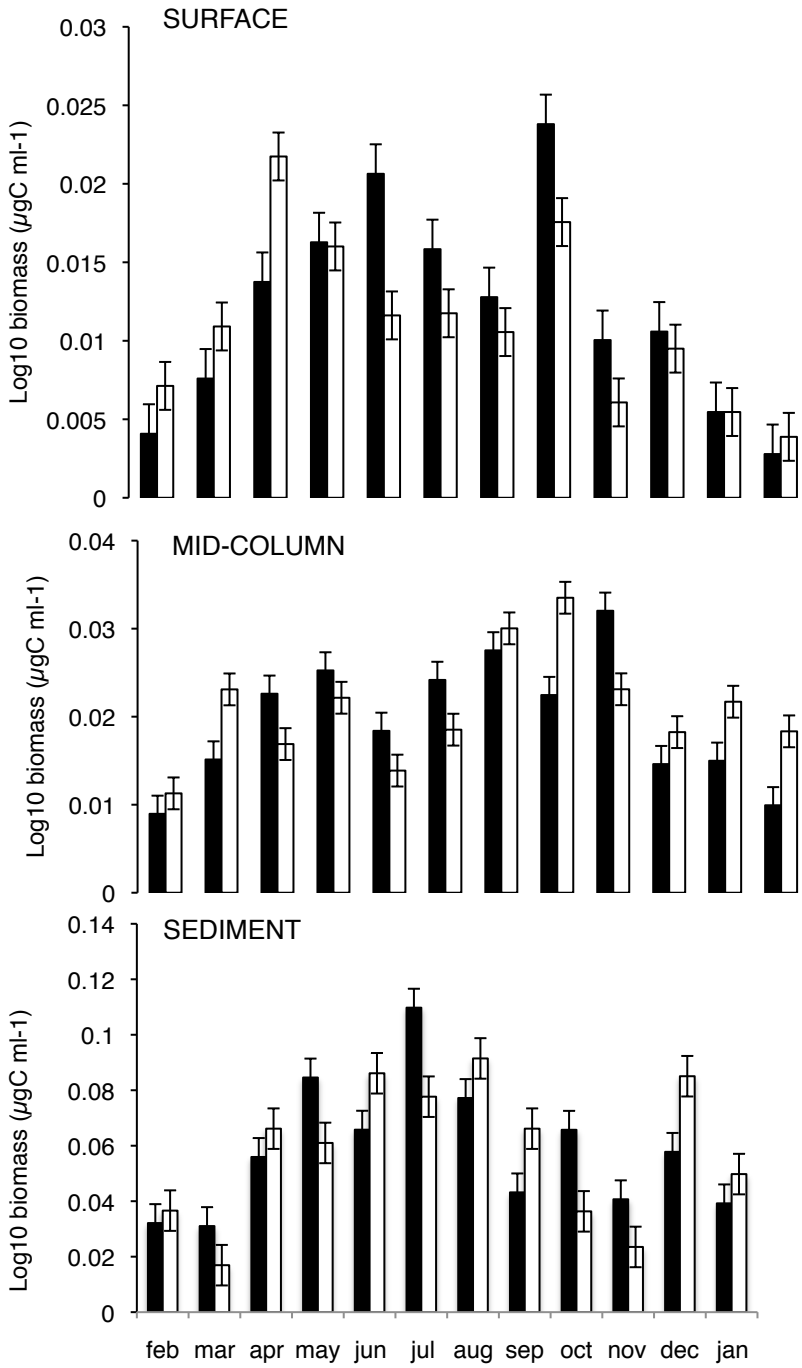


Figure 4.8 Log_{10} biomass (μgC ml^{-1}) (±1SE) for the heterotrophic protozoa (heterotrophic nanoflagellates, ciliates and amoebae combined, per pond and averaged per treatment) in the warmed (black bars) and ambient (white bars) mesocosms, across the sampling period February 2009 to January 2010.

MEIOFAUNA

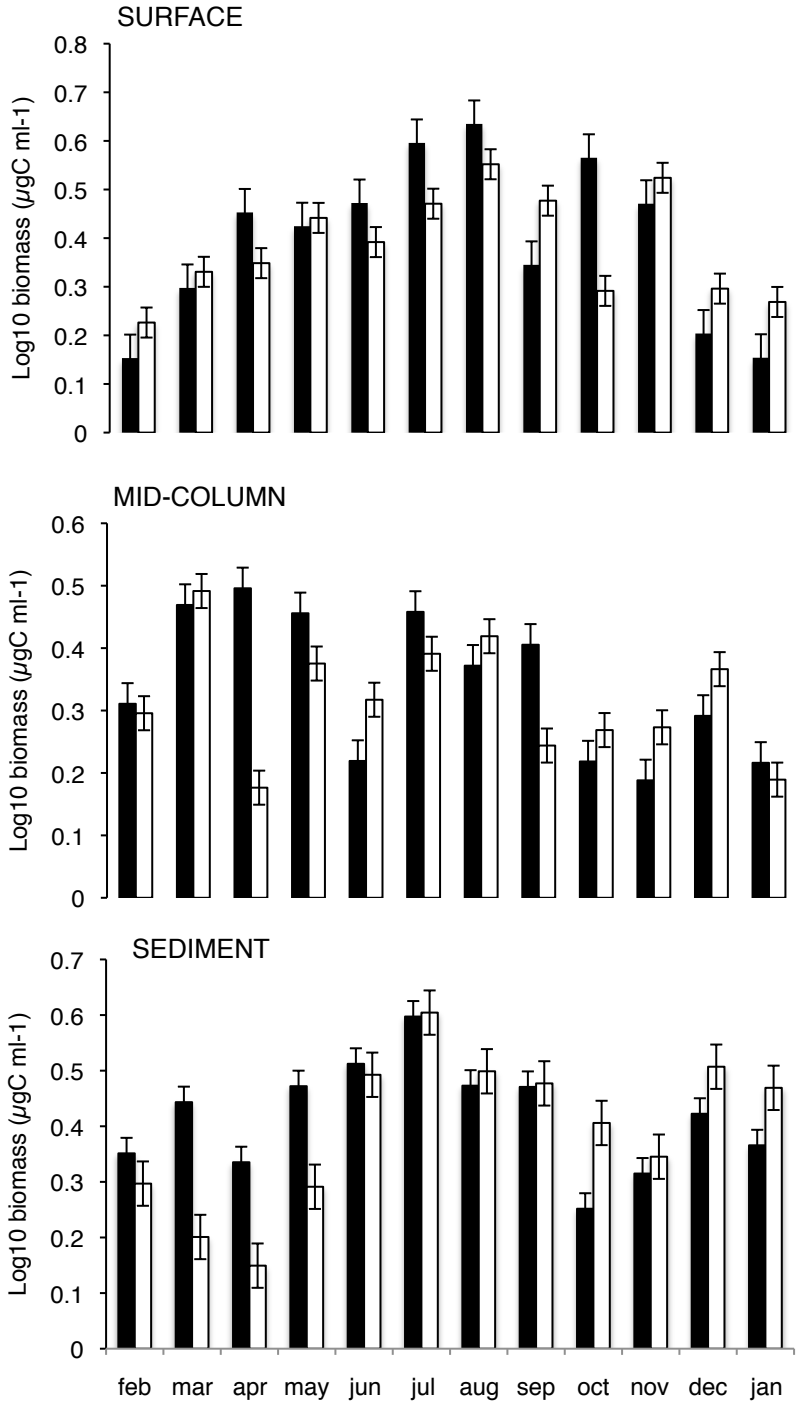


Figure 4.9 Log_{10} biomass (μgC ml^{-1}) (±1SE) for the meiofauna in the ambient (black bars) and warmed (white bars) mesocosms at each depth in the water column across the sampling period from February 2009 to January 2010.

Table 4.2 RMANOVA results for the algae, for both abundance and biomass. Resutls for all algae (diatoms and desmids) examined in the ponds across the whole sampling period from February 2009 to January 2010.

	ABUNDANCE		**BIOMASS**	
Variable/Model	**F-stat**	**p-value**	**F-stat**	**p-value**
Main effect: treatment	$F_{1,19}$=9.8	0.08	$F_{1,19}$=32.42	0.06
Main effect: Month	$F_{1,119}$=37.9	**0.011**	$F_{1,119}$=30.6	**0.013**
Main effect: Depth	$F_{1,59}$=8.12	0.09	$F_{1,59}$=43.4	0.029
Interaction effect (two-way) Log10abundance~ treatment x month	$F_{1,99}$=51.2	**0.006**	$F_{1,23}$=41.6	**0.023**
Interaction effect (two-way) Log10 abundance~ treatment x depth	$F_{1,21}$=19.9	**0.031**	$F_{1,23}$=53.21	**0.017**

Table 4.3 RMANOVA results for the flagellates showing main effects and interaction terms for the abundance and biomass of the communities in ambient and warmed mesocosms, across the sampling period from February 2009 to January 2010.

	ABUNDANCE		**BIOMASS**	
Variable/Model	**F-stat**	**p-value**	**F-stat**	**p-value**
Main effect: treatment	$F_{1,19}$=23.62	**0.035**	$F_{1,19}$=3.21	0.08
Main effect: Month	$F_{1,119}$=12.1	**0.009**	$F_{1,119}$=19.6	**0.03**
Main effect: Depth	$F_{1,59}$=3.68	0.07	$F_{1,59}$=29.2	0.074
Interaction effect (two-way) Log10abundance~ treatment x month	$F_{1,99}$=41.2	**0.012**	$F_{1,23}$=3.56	0.067
Interaction effect (two-way) Log10 abundance~ treatment x depth	$F_{1,21}$=9.9	0.08	$F_{1,23}$=24.9	**0.017**
Interaction effect (three-way) Log10 abundance~ treatment x depth x month	$F_{1,198}$=34.68	**0.016**	$F_{1,23}$=39.18	**0.021**

Table 4.4 RMANOVA results for the heterotrophic protists across the sampling period February 2009 to January 2010

	ABUNDANCE		BIOMASS	
Variable/Model	**F-stat**	**p-value**	**F-stat**	**p-value**
Main effect: treatment	$F_{1,19}$=2.68	0.072	$F_{1,19}$=8.21	0.056
Main effect: Month	$F_{1,119}$=56.23	**0.008**	$F_{1,119}$=10.6	**0.001**
Main effect: Depth	$F_{1,59}$=23.12	0.029	$F_{1,59}$=43.4	0.07
Interaction effect (two-way) Log10abundance~ treatment x month	$F_{1,99}$=43.22	**0.03**	$F_{1,23}$=52.1	**0.001**
Interaction effect (two-way) Log10 abundance~ treatment x depth	$F_{1,21}$=29.4	**0.003**	$F_{1,23}$=30.9	**0.017**
Interaction effect (three-way) Log10 abundance~ treatment x depth x month	$F_{1,198}$=34.68	**0.006**	$F_{1,23}$=25.9	**0.004**

Table 4.5 RMANVOA results for the meiofauna, for abundance and biomass. There were no significant three-way interactions so the model was excluded from the table.

	ABUNDANCE		BIOMASS	
Variable/Model	**F-stat**	**p-value**	**F-stat**	**p-value**
Main effect: treatment	$F_{1,19}$=13.46	0.055	$F_{1,19}$=2.42	0.07
Main effect: Month	$F_{1,119}$=12.1	**0.009**	$F_{1,119}$=30.6	**0.001**
Main effect: Depth	$F_{1,59}$=23.12	0.029	$F_{1,59}$=.4	**0.029**
Interaction effect (two-way) Log10variable~ treatment x month	$F_{1,99}$=33.8	**0.021**	$F_{1,23}$=7.56	**0.03**
Interaction effect (two-way) Log10 variable~ treatment x depth	$F_{1,21}$=19.9	**0.025**	$F_{1,23}$=27.53	**0.017**
Interaction (two-way) Log10 variable~ month x depth	$F_{1,21}$=19.9	**0.043**	$F_{1,23}$=27.53	0.07

4.5 Discussion

There is evidence that ecological responses to recent climate change are already occurring at the species (and therefore, population) level (Walther *et al.* 2002; Parmesan 2006; Walther 2010), but scaling from populations to communities and ecosystems is challenging because of the perceived indeterminacy of ecological interactions (Yodzis and Innes 1992; Montoya *et al.* 2006; Woodward *et al.* 2010). The use of small organisms in experimental mesocosms offers an effective means of testing warming theories, such as the TSR, on an intergenerational scale and in controlled and replicated arenas. TSR posits a smaller resultant body size due to an increased rate of development where individuals will reach a smaller adult size, having developed to adult stage more rapidly, driven by higher temperatures. Whilst I did not measure development rate in this study, individual body size was measured and converted into biomass.

In this chapter, I investigated the impact of warming on the abundance and biomass of microbial-meiofaunal assemblages along spatial and temporal gradients, to address the following hypotheses: (i) does warming have an effect on the overall abundance and biomass of microbial taxa (ii) is this difference classified as a shift in phenology, given by a single, significant interaction term between treatment and sampling month and (iii) warming will also impact spatial patterns, made evident by a significant interaction between treatment and location in the water column (i.e. depth).

Warming did not have a significant effect on the overall abundance and biomass of the groups examined [*hypothesis (i)* unsupported]. However, there was evidence for a shift in phenology over the annual cycle, indicated by a

significant interaction between treatment and sampling month in all the communities examined [*hypothesis (ii)*] as well as significant two-way and three-way interactions between treatment, month and sample depth in the heterotrophic protists which include the ciliates, heterotrophic flagellates and amoebae [*hypothesis (iii)*].

The implications of the interaction terms (warming x season x depth) for food webs and energy flow from these basal levels to the higher levels of organization (e.g. fish and subsequently humans), are far reaching due to the varied roles that these small organisms play in natural systems, acting as; (1) food sources for higher organisms, (2) drivers of key processes (in particular the photosynthetic groups which include desmids, diatoms and autotrophic flagellates) and thus, (3) provide an important link between basal species and ecosystem processes (e.g detoxification of water resources) that humans depend upon for provisioning services. Further research is required to better link structural changes at the community and population level to functional changes in natural systems (Dossena *et al.* 2012). The use of metagenomics (e.g. He *et al.* 2010), quantitative PCR and pyrosequencing methods (e.g. Sheik *et al.* 2011) could advance studies like this one by increasing the precision with which microbial composition is quantified. For example, Sheik *et al.* (2011) used PCR and 454-pyrosequencing to investigate the effect of warming and drought on in grassland microbes. They showed definitively that warming interacted with drought to produce marked shifts in the abundance and community composition.

Impacts of phenological changes in the microbial loop

Phenological changes – the timing of seasonal activities e.g. flowering time and breeding time have advanced (Walther *et al.* 2002) and that these changes are due to climate change (Hughes 2000). Here, I defined phenology within the microbial loop prganisms examined in this study, as the peaks in abundance and biomass of the microbial loop taxa examined, are a predictable response of various species to warming and not just confined to the microbial loop, being observed in many species of plant and animal across the globe (Parmesan and Yohe 2003; Parmesan 2006). Phenological changes are relatively easy to measure and have been used to demonstrate the effects of global warming in many species, from plants, vertebrates and invertebrates (Visser and Both 2005).

I have shown that shifts in phenology are also apparent within 4 major taxonomic groups of the microbial loop – with elevated abundance of the major taxa of the microbial loop deviating from that of the ambient seasonal cycle (i.e. warming has a different effect in different seasons). In addition to this, warming has different effects in different seasons and at different depths for some, but not all of the taxa. If the phenology of a species is changing at a different rate to that of species upon which it depends on for food or which constitute its ecological conditions, there is potential for mismatch of seasonal activities. This fits in with the general finding that there are different rates of change in the phenology of plants, insects, vertebrates (Parmesan and Yohe 2003; Voigt et al. 2003), leading to the mismatch. In the microbial loop, in particular, this has implications for nutrient cycling and energy flow at higher levels of organisation.

Caveats

Mesocosm studies are an abstract approximation of natural ecosystems, but do allow ecologists to isolate the effects of temperature from other potentially confounding variables (e.g. latitudinal and biogeographical effects) while studying entire replicated communities (e.g. Yvon-Durocher *et al.* 2010). The results of this may be especially valuable as it is essential that ecologists explore the effects of the main components of climate change (e.g. warming) on community structure and attempt to link findings to ecosystem functioning (Tylianakis *et al.* 2008; Montoya and Raffaelli, 2010). Models resulting from such studies can then be applied to natural systems to examine potential concordance in underlying patterns.

Conclusion

In this study, I isolated the effects of warming on the community composition of the microbial, focussing specifically on the abundance and biomass of 4 major groups with greater resolution that in *chapter 3* of this thesis. I found no overall significant effect of temperature on either the total abundance or the biomass of the communities tested. However, I have shown that there was a subtle effect in terms of interactions with the sampling month and with the depth of the sample. Rapidly rising temperatures therefore have the potential to alter the structure of these communities *via* indirect effects with temporal and spatial gradients which may affect the rates of the key ecosystem processes that microbial communities mediate such as nutrient cycling and decomposition (e.g. Hooper *et al.* 2002; Reiss *et al.* 2009). However, this connection between structure and function is still not explicit so furthering our understanding of the more subtle effects of temperature on these organisms and their role in food webs, will be an

important part of making future predictions about the health of freshwater systems under global warming.

4.6 References

Alheit, J. and Scheibel, W. (1982) Benthic harpacticoids as a food source for fish *Marine Biology*, **70,** 141-147

Atkinson, D. (1994) Temperature and organism size—A biological law for ectotherms *Adv. Ecol. Res.* **25**, 158

Atkinson, D. (1995) Effects of temperature on the size of aquatic ectotherms: Exceptions to the general rule *Journal of Thermal Biology* **20**, 61-74

Beaver, J.R. and Crisman, T.L. (1989) The role of ciliated protozoa in pelagic freshwater ecosystems. *Microbial Ecology*, **17**, 111–136

Bergmann, C. (1847) Uber die Verhaltnisse der Warmeokonomie der Thiere zu ihrer Grosse*Gottinger Studien* **3**, 595–708

Beveridge, O.S., Humphries, S., and Petchey, O.L. (2010) The interacting effects of temperature and food chain length on trophic abundance and ecosystem function *J. Anim. Ecol.* **79**, 693–700

Bick, H. (1972) Ciliated Protozoa – an Illustrated Guide to the Species Used as Biological Indicators in Freshwater Biology, *World Health Organization, Geneva*

Brown, J.H., Gillooly, J.F., Allen, A.P., Savage, V.M.and West, G.B. (2004) Toward a metabolic theory of ecology *Ecology* **85,** 1771-1789

Carrick H.J. and Fahnenstiel G.L. (1990) Planktonic protozoa in lakes Huron and Michigan – seasonal abundance and composition of ciliates and dinoflagellates. *Journal of Great Lakes Research*, **16**, 319–329

Carrick, H.J., Fahnenstiel, G.L., Stoermer, E.F. and Wetzel, R.G. (1991) The importance of zooplankton-protozoan trophic couplings in lake Michigan *Limnology and Oceanography*, **36**, 1335–1345

Carpenter, S.R. (1996) Microcosm experiments have limited relevance for community and ecosystem ecology *Ecology* **77**, 677–680

Chinnasamy, S., Ramakrishnan, B., Bhatnagar, A. and Das, K.C. (2009) Biomass Production Potential of a Wastewater Alga *Chlorella vulgaris*ARC 1 under Elevated Levels of CO_2 and Temperature *Int. J. Mol. Sci.* **10**, 518-532

Corliss, J.O. (1979) The Ciliated Protozoa – Characterization, Classification and Guide to the Literature *Pergamon Press, Oxford*

Curds, C.R., Gates, M.A. and Roberts, D.M. (1982) British and Other Freshwater Ciliated Protozoa. Part I. Ciliophora: Kinetofragminophora*Cambridge University Press, Linnean Society of London, London.*

Curds C.R., Gates M.A. and Roberts D.M. (1983) British and Other Freshwater Ciliated Protozoa. Part II. Ciliophora: Oligohymenophora and Polyhymenophora. *Cambridge University Press, Linnean Society of London, London*

Damuth, J. 1981 Population-density and body size in mammals *Nature* **290**, 699-700

Damuth, J. (1987) Interspecific allometry of population density in mammals and other animals: The independence of body mass and population energy-use*Biol. J. Linn. Soc.* **31**, 193–246

Delong, J.P., Okie, L.G., Moses, M.E., Silby, R.M. and Brown, J.H. (2010) Shifts in metabolic scaling, production and efficiency across major evolutionary transitions of life *PNAS,* **107:29**, 12941-12945

Dossena, M., Yvon-Durocher, G., Grey, J., Montoya, J.M., Perkins, D.M., Trimmer, M., and Woodward, G. (2012) Warming alters community size structure and ecosystem functioning *Proc. R. Soc. B.* **279**, 3011-3019

Ekelund, F. and Rønn (1994) Notes on protozoa in agricultural soil, with emphasis on heterotrophic flagellates and naked amoebae and their ecology *FEMS Microbiology Reviews* **15**, 321-353

Elton, C. (1927) Animal Ecology *Sidgwick and Jackson, London*

Fenchel, T. (1975) The quantitative importance of the benthic microfauna of an Arctic tundra pond. *Hydrobiologia*, **46**, 445–464.

Fenchel, T. (1987) *Ecology* of Protozoa – the Biology of Free- Living Phagotrophic Protists. Springer-Verlag, New York.

Finlay B.J., Clarke K.J., Cowling A.J., Hindle R.M., Rogerson A. and Berninger U.G. (1988) On the abundance and distribution of protozoa and their food in a productive fresh-water pond *European Journal of Protistology* **23**, 205–217

Finlay B.J., Tellez, C. and Esteban, G. (1993) Diversity of free-living ciliates in the sandy sediment of a Spanish stream in winter *Journal of General Microbiology* **139**, 2855–2863

Finlay, B.J., Maberly, S.C. and Esteban, G.F. (1996) Spectacular abundance of ciliates in anoxic pond water: contribution of symbiont photosynthesis to host respiratory oxygen requirements *FEMS Microbiology Ecology* **20,** 229-235

Finlay B.J., Maberly S.C. and Cooper J.I. (1997) Microbial diversity and ecosystem function*Oikos*, **80**, 209–213

Finlay*, B.J* and Esteban, G.F (1998) *Freshwater protozoa: biodiversity and ecological function Biodiversity and Conservation **7, 1163-1186***

Foissner, W., Blatterer, H., Berger, H. and Kohmann, F. (1991) Taxonomische und o kologische Revision der Ciliaten des Saprobiensystems – Band I:

Cyrtophorida, Oligo- trichida, Hypotrichia, Colpodea*Informationsberichte Des Bayerischen Landesamtes fur Wasserwirtschaft,* **1**, 1–478

Foissner W., Berger H. and Kohmann F. (1992) Taxonomische und okologische Revision der Ciliaten des Saprobiensystems – Band II: Peritrichia, Heterotrich- ida, Odontostomatida*Informationsberichte Des Bayeris- chen Landesamtes fur Wasserwirtschaft*, **1,** 1–502

Foissner, W., Berger, H. and Kohmann, F. (1994) Taxonomischeund okologische Revision der Ciliaten des Saprobiensystems – Band III: Hymenostomata, Prosto- matida, Nassulida*Informationsberichte Des Bayerischen Landesamtes für Wasserwirtschaft,* **1**, 1–548

Foissner, W., Berger, H., Blatterer, H. and Kohmann, F. (1995) Taxonomische und okologische Revision der Ciliaten des Saprobiensystems – Band IV: Gymnostomatea, Loxodes, Suctoria*Informationsberichte Des Bayerischen Landesamtes fur Wasserwirtschaft* **1**, 1–540

Foissner, W. and Berger, H. (1996) A user-friendly guide tothe ciliates (Protozoa, Ciliophora) commonly used by hydrobiologists as bioindicators in rivers, lakes, and waste waters, with notes on their ecology *Freshwater Biology* **35**, 375–482

He ZL, Xu MY, Deng Y*, et al.* (2010) Metagenomic analysis reveals a marked divergence in the structure of belowground microbial communities at elevated CO_2*Ecology Letters,* **13**, 564-575

Forster, J., Hirst, A.G. and Atkinson, D. (2011) How do organisms change size with changing temperature? the importance of reproductive method and ontogenetic timing *Functional Ecology* **25**, 1024–1031

Hooper, D.U. *et al.* (2002) Species diversity, functional diversity, andecosystem functioning. *In Biodiversity and Ecosystem Functioning. Synthesis and Perspectives (Loreau, M. et al., eds),* pp. 195–208, *Oxford University Press*

IPCC (2007) in Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*Ed. Parry, M.L., Canziani, O.F., Palutikof, J.P., van der Linden, P.J. and Hanson, C.E. (Cambridge University Press, Cambridge) pp. 7-22*

Jacobsen, D., Schultz, R. and Encalada, A. (1997) Structure and diversity of stream invertebrate assemblages: the influence of temperature with altitude and latitude *Freshwater Biology* **38**: 247–261

James, F. C. (1970) Geographic Size Variation in Birds and Its Relationship to Climate *Ecology* **51**, 365-390

Kahl, A. (1930) Urtiere oder Protozoa I: Wimpertiere oder Ciliata (Infusoria) 1. Allgemeiner Teil und Prostomata*Tierwelt Deutschlands,***18**, 1–180

Kahl, A. (1931) Urtiere oder Protozoa I: Wimpertiere oder Ciliata (Infusoria) 2.

Holotricha ausser den im 1. Teil behandelten Prostomata *Tierwelt Deutschlands*, **21**, 181– 398

Kahl, A. (1932) Urtiere oder Protozoa I: Wimpertiere oder Ciliata (Infusoria) 3. Spirotricha*Tierwelt Deutschlands*, **25**, 399–650

Kahl, A. (1935) Urtiere oder Protozoa I: Wimpertiere oder Ciliata (Infusoria) 4. Peritricha und Chonotricha*Tierwelt Deutschlands*, **30**, 651–886

Kerr, S.R. and Dickie, L.M. (2001) The Biomass Spectrum: A Predator Prey Theory of Aquatic Production, *Columbia University Press, New York*

Miller, G.H., Fogel, M.L., Magee, J.W,Gagan, M.K., Clarke, S.J., Johnson, B.J. (2005) Ecosystem Collapse in PleistoceneAustralia and a Human Rolein Megafaunal Extinction *Science,* **309**, 287

Montoya, J.M., Pimm S.L., Sole, R.V. (2006) Ecological networks and their fragility. *Nature,* **442**, 259–264

Montoya, J.M. and Raffaelli,D. (2010) Climatechange,biotic interactions and ecosystem services*Phil. Trans. R. Soc. B.* **365**, 2013–2018

Moss, B., McKee, D., Atkinson, D., Collings, S.E., Eaton, J.W., Gill, A.B., Harvey, I., Hatton, K., Heyes, T., and Wilson, D. (2003) How important is climate? Effects of warming, nutrient addition and fish on phytoplankton in shallow lake microcosms *J. Appl. Ecol.* **40**, 782–792

Newsham, K.K., and Garstecki, T. (2007) Interactive effects of warming and species loss on model Antarctic microbial food webs *Funct. Ecol.* **21**, 577–584

O'Connor, M.I., Piehler M.F., Leech, D.M., Anton, A., Bruno, J.F. (2009) Warming and resource availability shift food web structure and metabolism *PLoS Biol* **7(8)**: e1000178. doi:10.1371/journal.pbio.1000178

O'Gorman, E.J., Pichler, D.E., Adams, G., Benstead, J.P., Craig, N., Cross, W.F., Demars, B.O.L., Friberg, N. Gísli Mar Gíslason[8], Rakel Gudmundsdóttir, R., Hawczak, A., Hood, J.M., Hudson, L.N., Liselotte Johansson, L., Johansson, M., Junker, J.R., Laurila, A., Manson, J.R., Mavromati, E., Nelson, D., Ólafsson, J.S., Perkins, D.M., Petchey, O.L., Plebani, M., Reuman, D.C., Rall, B.C., Stewart, R., Thompson, M.S.A. and Woodward, G. (2012) Impacts of warming on the structure and function of aquatic communities: individual- to ecosystem-level responses *An. Ecol. Rev. **47,*** 81-176

Parmesan, C. and Yohe, G. (2003) globally coherent fingerprint of climate change impacts across natural systems *Nature* **421**, 37-42

Parmesan, C. (2006) Ecological and evolutionary responses to recent climate change, *Annual Review of Ecology Evolution and Systematics,* **37,** 637-669

Pascual, M.M. and Dunne, J.A. (2005) Ecological networks: linking structure to dynamics *Oxford, UK: Oxford University Press*

Petchey, O., Mcphearson, P., Casey, T.and Morin, P. (1999) Environmental warming alters food-web structure and ecosystem function *Nature* **402,** 69-72

Petchey, O. L., Beckerman, A. P., Riede, J. O., and Warren, P. H. (2008) Size, foraging, and food web structure *Proceedings of the National Academy of Sciences of the United States of America* **105**, 4191-4196

Peters, R.H. (1983) The ecological implications of body size Cambridge University Press, Cambridge

Purdy, K. J., Hurd, P. J., Moya-Larano, J., Trimmer, M., Oakley, B. B., and Woodward, G. (2010) Systems biology for ecology: from molecules to ecosystems*inG. Woodward, editor. Integrative Ecology: From Molecules to Ecosystems* pp.87-149

Reiss, J.and Schmid-Araya, J.M. (2008) Existing in plenty: abundance, biomass and diversity of ciliates and meiofauna in small streams*Freshwater Biology*, **53,** 652-668

Reiss, J., Forster, J., Cassio, F., Pascoal, C., Stewart, R., Hirst, A.G. (2010) When Microscopic Organisms Inform General Ecological Theory Ed: Woodward, G.*Integrative Ecology: From Molecules to Ecosystems Book Series: Advances in Ecological Research* **43,** p. 45-85

Reuman, D.C., Mulder, C., Raffaelli, D., and Cohen, J.E. (2008) Three allometric relations of population density to body mass: theoretical integration and empirical tests in 149 food webs. *Ecology Letters***11**, 1216-1228

Schmid-Araya, J.M. (1994) Temporal and spatial distribution of benthic microfauna in sediments of a gravel streambed *Limnology and Oceanography*, **39**, 1813–1821

Schmid-Araya, J.M. (1997) Temporal and spatial dynamics of meiofaunal assemblages in the hyporheic interstitial of a gravel stream. In: Groundwater/Surface Water Ecotones: Biological and Hydrological Interactions and Management Options (Eds J. Gibert, J. Mathieu and F. Fournier), pp. 29–36 *Cambridge University Press*, Cambridge

Sheik, C.S., Beasley, W.H., Elshahed, M.S., Zhou, X., Luo, Y., Krumholz, L.R. (2011) Effect of warming and drought on grassland microbial communities*The ISME Journal* **5**, 1692–1700

Sheridan, J.A. and Bickford, D. (2011) Shrinking body size as an ecological response to climate change *Nature Climate Change* **1**, 401-406

Smetacek, V. (1981) Annual cycle of protozooplankton in the Kiel Bight *Marine Biology* **63**, 1–11

Stoecker, D.K. and Capuzzo, J.M. (1990) Predation on protozoa: its importance to zooplankton *J. Plankton Res.* **12**, 891–908

Steffen, W., Crutzen, P.J., and McNeill, J.R. (2007) The Anthropocene: are humans now overwhelming the great forces of nature*AMBIO: A Journal of the Human Environment* **36**, 614-621

Swan, C.and Palmer, M.A. (2000) What drives small-scale spatial patterns in lotic meiofauna communities? *Freshwater Biology* **44,** 109-121

Tylianakis, J.M., Didham, R.K., Bascompte, J., Wardle, D.A. (2008) Global change and species interactions in terrestrial ecosystems *Ecology Letters* **11**, 1351–1363

Visser, M.E., Both, C. (2005) Shifts in phenology due to global climate change: the need for a yardstick. *Proc. Roy. Soc. B,* **272,** 2561-2569.

Walters, R.J., Hassall, M., (2006) The temperature-size rule in ectotherms: may a general explanation exist after all? *American Naturalist* **167**: 510–523

Walther, G.R., Post, E., Convey, P., Menzel, A., Parmesan, C., Beebee, T.J.C., Fromentin, J.M., Hoegh-Guldberg, O. and Bairlein, F. (2002) Ecological responses to recent climate change *Nature* **416,** 389-395

Walther, G.R. (2010) Community and ecosystem responses to recent climate change*Philosophical Transactions of the Royal Society B: Biological Sciences* **365**, 2019-2024

Winder, M. and Schindler D.E., (2004) Climate change uncouples trophic interactions in an aquatic ecosystem *Ecology* **85**: 2100– 2106

Winder, M., Reuter, J.E., Schladow, S.G. (2009) Lake warming favours small-sized planktonic diatom species *Proceedings of the Royal Society B-Biological Sciences,* **276**, 427-435

Woodward, G.*, Perkins. D.M., Brown, L., (2010b)* Climate change and freshwater ecosystems: impacts across multiple levels of organization *Phil. Trans. R. Soc. B***. 365:1549,** 2093-2106

Yodzis, P., Innes, S. (1992) Body size and consumer-resource dynamics *American Naturalist* **139,** 1151–1175

Yvon-Durocher, G., Jones, J.I., Trimmer, M., Woodward, G., and Montoya, J.M. (2010a) Warming alters the metabolic balance of ecosystems. *Philosophical Transactions of the Royal Society B-Biological Sciences* **365**, 2117-2126

Yvon-Durocher, G., Trimmer, M., Woodward, G., and Montoya, J.M. (2010b) Warming alters the size spectrum and the distribution of body size in aquatic ecosystems. *Global Change Biol.* **17**, 1681-1694

Chapter 5

Population-level shifts in size and abundance of protists and meiofauna in response to environmental warming

5.1 Abstract

Organisms of the microbial loop, such as the protoza and meiofauna, play a key role in important processes in aquatic systems, yet we know comparatively little about how predicted levels of warming might impact the structure of their populations in natural aquatic systems.

I used 20 experimental mesocosms, 10 of which were heated between 3-5°C, to simulate global warming on aquatic freshwater systems. The aim was to investigate the effect of warming on several populations of important microbial loop species, including 2 autotrophic protist genera (*Closterium spp.* and *Peridinium spp.*) and 2 heterotrophic protists genera (a genus of ciliate, *Halteria spp.*) and a rotifer (*Keratella spp.)*. Over a 12-month sampling period, I recorded the abundance and body mass of these microbial-meiofaunal populations and tested the following hypotheses; (1) Warming will result in a larger number of smaller individuals in the warmed mesocosms, shown by a decrease in the individual body sizes at the population level, in warmed mesocosms, compared to the ambient ones. (2) Altered abundance and biomass of the study populations as a results of warming will be observable at this level of

organisation (3) There will be significant two- and three-way interactions between treatment, month and depth as evidence for changes in phenology (in terms of abundance and biomass), for each population.

Results showed that population shifts were evident at certain times of the year (summer and winter) in all the genera examined, as indicated by significant interactions between temperature and month and depth. As a result of the decrease in mean body size at the population level, population biomass was lower despite an increase in abundance in warmed mesocosms.

The implications of reduced abundance and/or biomass in populations of microbial loop organisms is complex as the same effects and patterns were not evident at the level of the whole community in *chapters 3 and 4* of this thesis, The implications of this are, that whilst some species populations are affected by warming, in terms of reduced body size, there may be other groups that remain unaffected. The resultant overall effect that temperature may be masked at the population level or that the microbial loop is much more plastic than previous research suggests.

5.2 Introduction

Anthropogenic environmental change is occurring at an unprecedented rate and is affecting biodiversity and the ecological services that species provide to humanity e.g. nutrient cycling, decomposition and carbon sequestration (Parmesan and Yohe 2003; Parmesan 2006; Russell *et al.* 2009). Direct impacts of anthropogenic climate change on the Earth's biota have been documented globally, on every continent, in every ocean and for every major taxon (Parmesan 2006). Common responses to climate change include range

shifts (e.g. Walther *et al.* 2002), phenological shifts (e.g. Root *et al.* 2003) and more recently, a shift in body size, with warmer temperatures favouring smaller bodied individuals (Angiletta and Dunham 2004; Daufresne *et al*. 2009, see *chapters 1-3* of this thesis).

Many of the critical ecosystem processes and services on which humans depend are mediated by the group of small organisms (including protists and meiofauna), within the microbial loop (Finlay and Esteban 1998; Finlay 2002; Fenchel 2008; Reiss and Schmid-Araya 2008), yet, with the exception of diatoms, still very little is known about their responses to environmental change in natural systems or large-scale field experiments (Wetzel 1983; Beaver and Crisman 1989; Winder *et al*. 2009; Reiss and Schmid-Araya 2008). Autotrophic protists (e.g. desmids, diatoms and phototrophic flagellates) are responsible for the bulk of primary production in many aquatic environments (O'Connor et al 2009; Winder *et al.* 2009); protozoan grazers transfer the production of algae and that of the bacteria that grow on algal exudates to higher trophic levels in the food chain. Protozoan non-grazers (i.e. bacterivores and predatory species) also form an integral part of the microbial loop and provide a link to higher levels of organization by acting as food sources for, meiofauna, macroinvertebrates and fish (e.g. Yozzo and Smith 1995). As such, these microbial organisms play a key role in, among other processes, energy transfer, carbon sequestration, ecosystem metabolism and nutrient cycling at the base of the food web in many ecosystems [see *chapter 1* (Sherr and Sherr 1994; Finlay and Esteban 1998; Hakenkamp and Morin 2000, 2002; Stead *et al.* 2003)]. Changes at the lower levels of biological organisation (individuals, populations) and within the microbial loop can have important consequences at higher, multispecies, levels

[(communities, food webs, ecosystems), see *chapter 1* of this thesis] through species-species interactions (grazing, predation) and interaction between species and abiotic factors (e.g. with temperature) (Hakenkamp and Morin 2000; Beveridge *et al.* 2010). It is therefore essential to further our understanding of the impact of global warming on population attributes of microbial loop organisms [standing stocks (e.g. abundance and biomass)] and also on population dynamics, which I investigate, using laboratory microcosms in *chapter 6* of this thesis.

In addition to playing a significant role in natural aquatic systems, protists and small metazoans have been useful in the experimental context to inform global ecological problems such as global warming, by the use of microcosms (Petchey *et al.*1999) and mesocosms (Benton *et al.* 2007; Montagnes *et al.* 2002; Reiss *et al.* 2010). The likely impacts of environmental warming, as a key component of predicted climate change, are especially poorly understood – most studies have used either laboratory microcosms (Petchey *et al.* 1999) or correlational survey data to assess population or community level change, while very few have measured ecosystem properties or used field mesocosms (but see Yvon-Durocher *et al.* 2010 a,b,c; Dossena et al. 2012). Such a system provides greater realism than microcosms yet maintains a high level of control so causality can be determined explicitly compared to field surveys which tend to be purely correlational (Jacobsen *et al.* 1997).

The population attributes I focus on in this chapter are population abundance and body size of individuals within populations of common (most abundant) protists and one common rotifer genus, identified from the mesocosms, over the course of one year (introducing a seasonal gradient) which will also identify

possible phenological responses of the chosen populations of these small organisms to warming, made evident in analyses by an interaction of treatment with sampling month. A few studies have considered the seasonal dynamics of protists and meiofauna in ponds (Carrick and Fahenstiel 1990; Carrick et al 1991; Gasol et al. 1990; Finlay and Esteban 1998). In Lake Wahsington, Winder and Schlindler (2004) identified phenological shifts in two species of zoolplankton; *Keratella cochlearis* (a genus I will focus on here) and *Daphnia pulicara*, that feed on phytoplankton (in particular, the diatom, *Astrionella formosa*). The timing of the diatom bloom has advanced over the past forty years (by 27 days), the emergence of *Keratella cochlearis* has also advanced by 21 days, in response to the diatom shift. *Daphnia pulicara* however, did not exhibit a phenological shift and is therefore mismatched with its predominant food source.

No existing studies that have addressed abundance and body mass in individual populations of protists and meiofauna in a controlled field experiment, to directly assess the impact of warming on the seasonal abundance and biomass throughout a whole seasonal cycle nor attempted to discern the seasonal dynamics of targeted taxa from the microbial loop. The microbial loop is complex in terms of energy pathways and the interactions that occur within the loop, so responses may be unique to the group as a whole (see *chapter 3* of this thesis) and coupled with cited difficulties in measuring and counting these small organisms, there has been little focus on them in food web and climate change ecology until recently (Reiss *et al.* 2010).

A common and key feature of protists and small metazoans is that of asexual reproduction and short generation times (usually hours) which makes

them useful model organisms for testing long-term effects of warming on population dynamics (see *chapter 6* of this thesis) and phenology due to rapid responses over many generations to environmental stressors like warming which has been shown in microcosms (e.g. Petchey *et al.* 1999; Delaney et al. 2003; Newsham and Garstecki 2007; Beveridge *et al.* 2010). Therefore, for the purpose of this study, this characteristic is useful and informative as it allows the observation of changes on an intergenerational scale.

Warming theories and body size

In some cases, a size-based approach may be more revealing than a purely taxonomy based approach to investigating the impacts of warming on microbial assemblages (Yvon-Durocher et al. 2010). Organism size often plays an important role in determining community structure and conveys information about the portioning of resources within a size-structured community (Damuth 1981,1987; Peters 1983; Brown *et al.* 2004; Petchey and Belgrano 2010). As yet, there are no general rules *per se* regarding the impact of warming on individuals however, a number of theories exist (see the summary table in *chapter 1*) that attempt to explain how warming will impact species, populations and communities by way of effects on individual organism body size. Briefly, (1) Bergman's rule refers to the latitudinal distribution of animals – smaller species tend to be found at warmer latitudes (Bergman 1847) and (2) James' rule states that, within a species, populations with smaller body size are generally found in warmer environments (James 1970, Atkinson 1994) and (3) the temperature–size rule (TSR) states that the individual body size of ectotherms tends to decrease with increasing temperature due to faster growth rates and lower final

size or a population age structure shift (Daufresne *et al.* 2009). These shifts may be due to a combination of direct (e.g. activation energies of biochemical reactions) and indirect (e.g. metabolic constraints) mechanisms. These hypotheses are also not mutually exclusive and an increase in smaller species at the community level (species shift hypothesis) may not necessarily be evident at higher levels of organisation (*chapter 1*).

The TSR is a subset of James's rule is a widely observed phenomenon within ectothermic species in which they grow more slowly at cooler temperatures and ultimately, reach a larger body size (Angilletta and Dunham 2003). It is a counter-intuitive phenomenon to classic life history theory that predicts smaller sizes in environments that retard growth although attempts have been made to explain this in terms of adaptive responses although is the subject of some debate (Angilletta and Dunham 2003); thermal constraints on cellular growth cause smaller sizes at higher temperatures. No single theory has been able to fully explain the mechanistic basis behind reduced body size so it is not known whether there is a general physiological mechanism causing the TSR or even if species share a similar pattern of thermal response across ontogeny. Attempts have been made to partition the effects in small organisms and discern separate mechanisms for unicellular and multicellular species separately (e.g. Forster *et al.* 2011) because there is a fundamental difference in the operation of the TSR between multicellular and unicellular organisms (Atkinson 2006; Forster *et al.* 2011a; Reiss *et al.* 2010) and suggesting that the existence of a general physiological mechanism is unlikely (Forster *et al.* 2011). In light of this, the possible mechanism could be related to a shift in life history stages and act at an intergenerational scale (see *chapter 6*).

I used a long-term mesocosm experiment to investigate the impact of warming on the mean individual body size, abundance and biomasss of four genera identified from the 20 mesocosms (1 desmid genus, 1 flagellate genus, 1 ciliateand 1 rotifer genus), during the sampling period. The aim was to describe the different taxa and their seasonal succession over the course of a sampling period of 12 months and to relate these to warming and attempt to discern the impact of warming on these populations at an intergenerational scale (hundreds of generations of protists). The experiment was used to test the following hypotheses:

(i) Warming will result in a larger number of smaller individuals, within genus, in the warmed mesocosms, shown by a decrease in the individual body sizes at the population level, in warmed mesocosms, compared to the ambient ones, in support of Jame's Rule (1970) that individuals will be smaller at higher temperatures [see *chapter 1*].

(ii) As a result of a decrease in mean body size at the population level, numerical abundances and biomass of the populations will be altered in warmed mesocosms compared to ambient mesocosms and similar patterns will be evident at the population level as for the whole community.

(iii) There will be significant two- and three-way interactions between treatment, month and depth as evidence for changes in phenology and emergence at the population level as an indirect effect of warming, as has been observed in earlier chapters, for the whole community (*chapter 3*) and at the community level (*chapter 4*).

5.3 Methods

Study Site

The experiment was part of an ongoing project set up in December 2006 at the Freshwater Biological Association River Laboratory, East Stoke, Dorset, UK. A detailed description of the experimental set-up is found in Yvon-Durocher *et al.* (2010 a,b) as well as in the study site chapter of this thesis. Briefly, it consisted of 20 freshwater mesocosms (~1m^3, 0.5m water depth): ten replicates were left at ambient temperature whilst the other 10 were warmed to 3.0 -5.0 °C (mean 4 °C) above ambient and had previously been seeded with benthic and pelagic taxa from the nearby river Frome and allowed to establish for a year before warming commenced.

Sampling

Samples of 10-ml were collected from each of the sediment, surface and mid-column of all 20 mesocosms, every month from February 2009 until March 2010 inclusive. Detailed sampling methods are found in the general methods section of this thesis (*chapter 2*). 1-ml sub samples were removed from each and placed in a Sedgwick rafter counting cell was used for counting and identification of live organisms, under light microscopy using an Olympus BX50 microscope at 40-400x magnification (Olympus Optical, Tokyo, Japan) (Finlay and Esteban 1998).

Identification and quantification

For the taxonomic assessment of the community composition of the microbial loop organisms present in the ponds, over the sampling period, individuals were counted and identified live within a 1-ml Sedgwick rafter counting cell, using the

following keys: Kahl (1930, 1931, 1932, 1935); Bick (1972); Corliss (1979); Curds, Gates and Roberts (1982, 1983); Foissner *et al.* (1991); Foissner, Berger and Kohmann (1992, 1994); Foissner *et al.* (1995).

Body size measurements

Live individuals were photographed and individual body sizes were measured using Image analysis software (Image J, Q Capture) under light microscopy using a Nikon SMZ-U stereomicroscope. Individual body dimensions (length and width) were converted to specific biovolume using common geometric formulae (Hobbie *et al.* 1997; Hillebrand *et al.* 1999). The biovolume of the protists (ciliates and flagellates) was converted into carbon content assuming 0.14-pgC μg^3(Putt and Stockner 1989; Reiss and Schmid Araya 2008). Meiofaunal biovolume was converted into individual body mass by assuming a specific gravity of 1.1 and individual carbon content was estimated assuming a dry/wet weight ratio of 0.25 and a dry carbon content of 40% (Feller and Warwick 1988; Reiss and Schmid Araya 2008).

Data Analysis

For individual body mass, the data from the three samples at each depth in mesocosms (*chapter 2*) were pooled and individual body mass was averaged across the mesocosms to obtain one value for each taxon per pond and plotted against time. Linear mixed effects (*lme*) models were applied to the data for each taxon to discern; (1) the effect of temperature on individual body mass and (2) the effect of possible two-way interactions (treatment x month and treatment x depth) on individual body size.

The numerical abundance and biomass of each population were compared between treatments by repeated measures analysis of variance (RMANOVAs). The raw data were log-transformed to meet assumptions of normality and homogeneity of variance. The RMANOVAs were performed separately at each depth. Depth was added into the model later on, to test for potential three-way interactions between (i.e. treatment x month x depth).

5.4 Results

During the study period, 1 dominant (the most abundant in their group) genus of desmid (*Closterium spp.*), 1 dominant autotrophic flagellate genus (*Peridinium spp.*), a ciliate genus, *Halteria spp.*) and 1 dominant rotifer genus (*Keratella spp.*) were identified in the ponds (see *appendices 1- 4* for taxa and species lists).

Closterium spp. (Desmidacea) and *Peridinium spp. (Peridinaceae)* are autotrophic protists and was found to be the most abundant within the major algae and flagellate taxa (more than 50% abundance within algae and flagellates, respectively, per sample) and throughout the sampling period.

Hypothesis (i) Population level shifts in individual body size

Treatment had a significant effect on average individual body size during some summer months for *Closterium spp.* (Figure 5.1) and the photosynthetic flagellate, *Peridinium spp.* (Figure 5.2). The protist *Halteria spp.* also showed a significant effect of warming on individual body size, across the sampling period – evident by a significant two-way interaction with treatment and month. The

rotifer, *Keratella spp.* showed very little variation in body size over the sampling period and was largely unresponsive to temperature. None of the groups exhibited significant variation in body size, between treatment and across the spatial gradient.

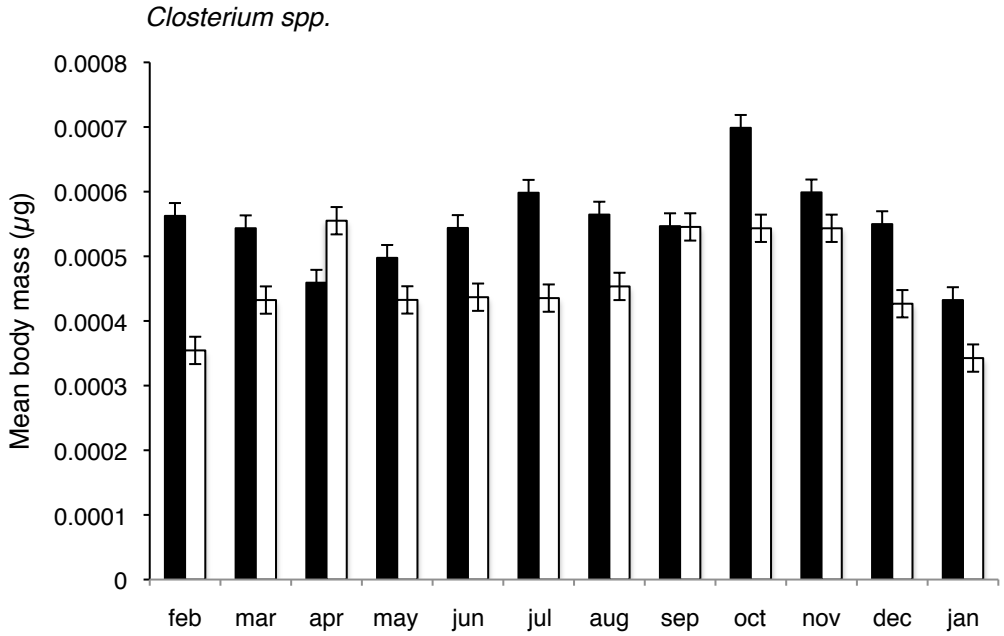


Figure 5.1 Mean body mass (±1SE) for the common desmid genus, *Closterium spp.*, across the sampling period February 2009 to January 2010. Black bars represent ambient mesocosms and white bars represent warmed mesocosms.

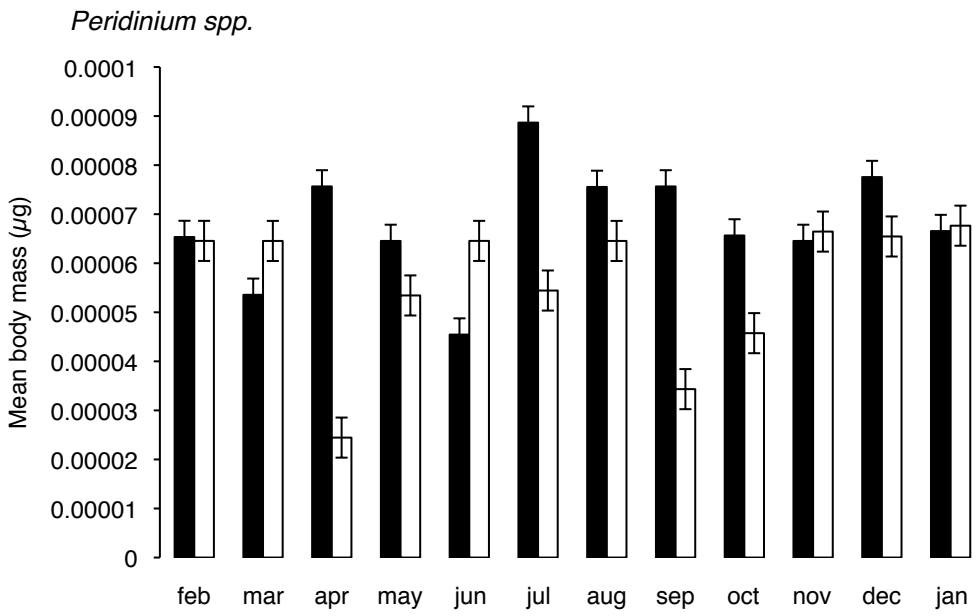


Figure 5.2 Mean body mass (±1SE) for the common flagellate genus, *Peridinium spp.,* across the sampling period February 2009 to January 2010. Black bars represent ambient mesocosms and white bars represent warmed mesocosms.

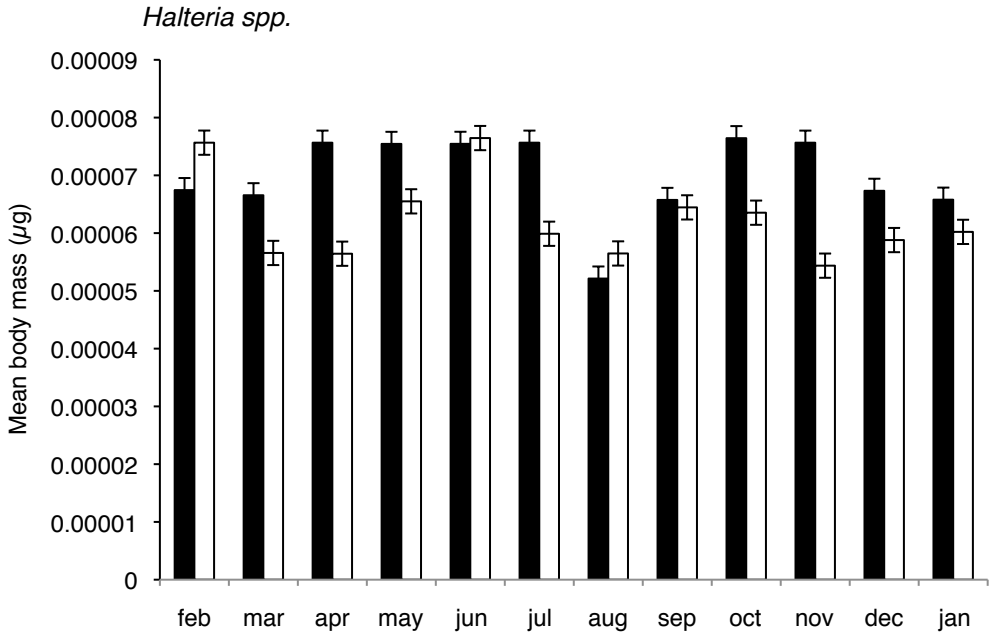


Figure 5.3 Mean individual body mass (±1SE) for the common ciliate genus *Halteria spp.,* across the sampling period February 2009 to January 2010. Black bars represent ambient mesocosms and white bars represent warmed mesocosms.

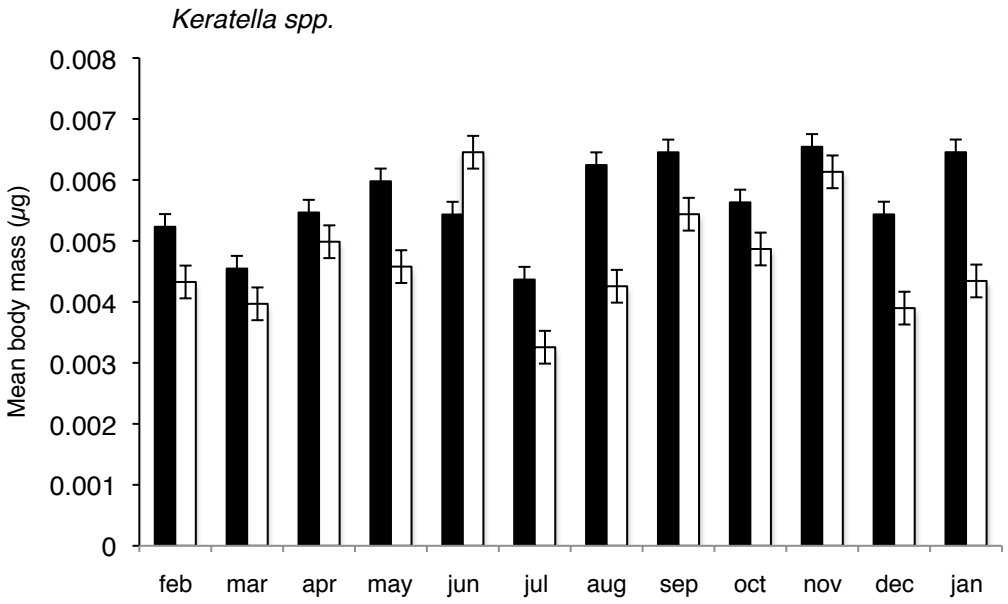


Figure 5.4 Mean individual body mass (±1SE) for the common rotifer genus *Keratella spp.* across the sampling period February 2009 to January 2010. Black bars represent ambient mesocosms and white bars represent warmed mesocosms.

Table 5.1 Results from linear mixed effects models, listed by genus for the effect of warming on the individual body mass of the dominant genera in the mesocosms. Each model was fitted with mesocosm as a fixed effect. Body mass was significantly affected by an interaction between the treatment and sample month for 3 out of the 4 genera tested.

Model	**Genus**	**F-ratio**	**P-value**
Log body mass~ treatment x month	*Closterium spp.*	$21.6_{1,22}$	**0.016**
	Peridinium spp.	$18.4_{1,22}$	**0.04**
	Halteria spp.	$22.9_{1,22}$	**0.002**
	Keratella spp.	$5.32_{1,22}$	0.07
Log body mass~ treatment x depth	*Closterium spp.*	$1.6_{1,5}$	0.54
	Peridinium spp.	$9.7_{1,5}$	0.048
	Halteria spp.	$12.1_{1,5}$	0.06
	Keratella spp.	$6.73_{1,5}$	0.004

Hypotheses (ii) and (iii) by genus: Population abundance and biomass

For *Closterium spp.*, warming did not have an overall significant effect on the abundance (Figure 5.3) or biomass (Figure 5.4) across the whole sampling period for populations of desmid [Table 5.2 (Abundance: RMANOVA $F_{1,19}$=13.70, p=0.0653; Figure 2. RMANOVA $F_{1,19}$ =11.43, p=0.0613; Biomass: RMANOVA $F_{1,19}$=16.9, p=0.082)].

In support of hypothesis (iii), there were significant two-way interactions between treatment and month and between treatment and depth (Table 5.2) for biomass only. There was also a significant three-way interaction for biomass (treatment x month x depth), supporting hypothesis (ii) that warming interacts with abiotic factors to influence population structure.

Table 5.2 RMANOVA results for *Closterium spp.* showing significant results of two-way and three-way ANOVAs for population abundances and biomass. Treatment was not significant overall due to switches in direction of influence during summer and winter months respectively.

	ABUNDANCE		**BIOMASS**	
Variable/Model	**F-stat**	**p-value**	**F-stat**	**p-value**
Main effect: treatment	$F_{1,19}$=9.17	0.073	$F_{1,19}$	0.041
Main effect: Month	$F_{1,119}$=59.1	**0.014**	$F_{1,119}$=10.6	**0.002**
Main effect: Depth	$F_{1,59}$=33.12	0.089	$F_{1,59}$=13.4	**0.029**
Interaction effect (two-way) Log10abundance~ treatment x month	$F_{1,99}$=5.56	**0.023**	$F_{1,23}$=17.56	0.052
Interaction effect (two-way) Log10 abundance~ treatment x depth	$F_{1,21}$=5.56	**0.0401**	$F_{1,23}$=19.56	0.062
Interaction effect (three-way)				
Interaction effect (three-way) Log10 abundance~ treatment x depth x month	$F_{1,198}$=45.5	0.09	$F_{1,23}$=25.51	0.072

For *Peridinium spp.*, warming did not have an overall (whole sampling period) significant effect on the abundance of the flagellate genus [Figure 5.5 (RMANOVA $F_{1,19}$, = 14.41, p=0.084) but population biomass was lower in warmed ponds than in ambient ponds indicating that warming [Figure 5.6 (RMANOVA $F_{1,19}$ =21.53, p=0.0125)].

In addition to the significant main effect of temperature, there was a significant two-way interaction between treatment and month, highlighting a potential shift in phenology in this genus, shown by shifts in peak abundance along the seasonal gradient. There was also a significant, 2-way interaction between treatment and depth. This is also evident from Figure 5.7, where the abundance of the flagellate shows more variation in warmed ponds compared to ambient ponds but peaks in abundance are not the same for each of the depth, for example, Peridinium shows a peak in abundance in April, in warmed ponds, in the sediment but a peak in abundance in May in the surface, in warmed ponds. In the mid-column, there is less dramatic fluctuation in peak abundance, with peak in abundance is in April, yet the whole seasonal cycle of abundance appears dampened by warming.

Table 5.3 RMANOVA results for *Peridinium spp.* showing significant results of two-way and three-way ANOVAs for population abundances and biomass. Treatment had a significant overall effect on the biomass of individuals sampled but not on the abundance of the population overall. There was a significant two-way interaction between treatment and month, indicative of the seasonal effect of warming and possible phenological changes.

	ABUNDANCE		**BIOMASS**	
Variable/Model	**F-stat**	**p-value**	**F-stat**	**p-value**
Main effect: treatment	$F_{1,19}$=8.37	0.084	$F_{1,19}$=21.53	**0.0125**
Main effect: Month	$F_{1,119}$=42.1	**0.01**	$F_{1,119}$=22.6	**0.001**
Main effect: Depth	$F_{1,59}$=13.12	0.089	$F_{1,59}$=43.4	**0.029**
Interaction effect (two-way) Log10abundance~ treatment x month	$F_{1,99}$=47.8	**0.003**	$F_{1,23}$=7.56	0.052
Interaction effect (two-way) Log10 abundance~ treatment x depth	$F_{1,21}$=20.1	0.0801	$F_{1,23}$=9.27	0.062
Interaction effect (three-way) Log10 abundance~ treatment x depth x month	$F_{1,198}$=27.6	0.09	$F_{1,23}$=5.91	0.051

For *Halteria spp.,* there was no overall effect of treatment on the abundance [Table 5.4 (Figure 5.7 RMANOVA $F_{1,19}$=13.46, p=0.0554)] or biomass [Table 5.4 (Figure 5.8 RMANOVA, $F_{1,19}$= 12.42, p=0.047), across the whole sampling year. There was evidence for significant two-way and three way interactions for this genus (Table 5.4). For this genus of protist, there was also no overall effect of depth, compared to the other genera, where the spatial gradient has a significant effect on the abundance – where organisms tend to be more

common in the sediment than in the mid-column and on the surface of the ponds.

Table 5.4 Main effects and interactive effects as obtained from an RMANOVA for *Halteria spp.*

	ABUNDANCE		**BIOMASS**	
Variable/Model	**F-stat**	**p-value**	**F-stat**	**p-value**
Main effect: treatment	$F_{1,19}$=13.46	0.055	$F_{1,19}$=12.42	0.047
Main effect: Month	$F_{1,119}$=12.1	**0.009**	$F_{1,119}$=10.6	**0.001**
Main effect: Depth	$F_{1,59}$=23.12	0.029	$F_{1,59}$=43.4	**0.029**
Interaction effect (two-way) Log10abundance~ treatment x month	$F_{1,99}$=51.2	**0.001**	$F_{1,23}$=7.56	**0.03**
Interaction effect (two-way) Log10 abundance~ treatment x depth	$F_{1,21}$=19.9	**0.031**	$F_{1,23}$=27.53	**0.017**
Interaction effect (three-way) Log10 abundance~ treatment x depth x month	$F_{1,198}$=34.68	**0.016**	$F_{1,23}$=39.18	**0.021**

For the rotifer genus, *Keratella spp.*, similarly to other small genera, temperature did not significantly influence abundance [Table 5.5, (Figure 5.7. RMANOVA $F_{1,19}$ =12.72, p=0.057); or biomass [Table 5.5, Figure 5.8 RMANOVA, $F_{1,19}$=11.72, p=0.0632) across the whole sampling period. However, as for the other genera, there were significant two-way interactions with treatment and depth for both abundance and biomass as well as a significant three-way interaction for abundance (Table 5.5).

In addition to changes in the mean abundance over time, warming seems to have a dampening effect on the whole seasonal cycle, where peaks in

abundance are generally lower for warmed mesocosms. There is a significant two-way interaction between treatment and month, showing that increased temperature works to change the peak abundance in the roifer genus, at different times of the year. There was also a significant two-way interaction between treatment and depth, showing that increased temperatures interact with the spatial gradient, accounting for variation in abundance at different depths. In addition to the two-way interaction, for *Keratella,* there is also a significant three-way interaction between treatment and depth and month, showing further complexity in terms of the effect of rising temperatures.

Table 5.5 RMANOVA results for *Keratella spp.* showing significant results of two-way and three-way ANOVAs for population abundances and biomass. Treatment was not significant overall, possibly due to switches in the direction of influence during summer and winter months respectively.

	ABUNDANCE		**BIOMASS**	
Variable/Model	**F-stat**	**p-value**	**F-stat**	**p-value**
Main effect: treatment	$F_{1,19}$=12.72	0.057	$F_{1,19}$=11.72	0.0632
Main effect: Month	$F_{1,119}$=16.47	**0.035**	$F_{1,119}$=17.2	**0.028**
Main effect: Depth	$F_{1,59}$=33.27	**0.029**	$F_{1,59}$=39.8	**0.018**
Interaction effect (two-way) Log10abundance~ treatment x month	$F_{1,99}$=24.41	**0.021**	$F_{1,23}$=28.56	**0.028**
Interaction effect (two-way) Log10 abundance~ treatment x depth	$F_{1,21}$=39.14	**0.031**	$F_{1,23}$=27.31	**0.027**
Interaction effect (three-way) Log10 abundance~ treatment x depth x month	$F_{1,198}$=34.68	**0.02**	$F_{1,23}$=16.29	0.071

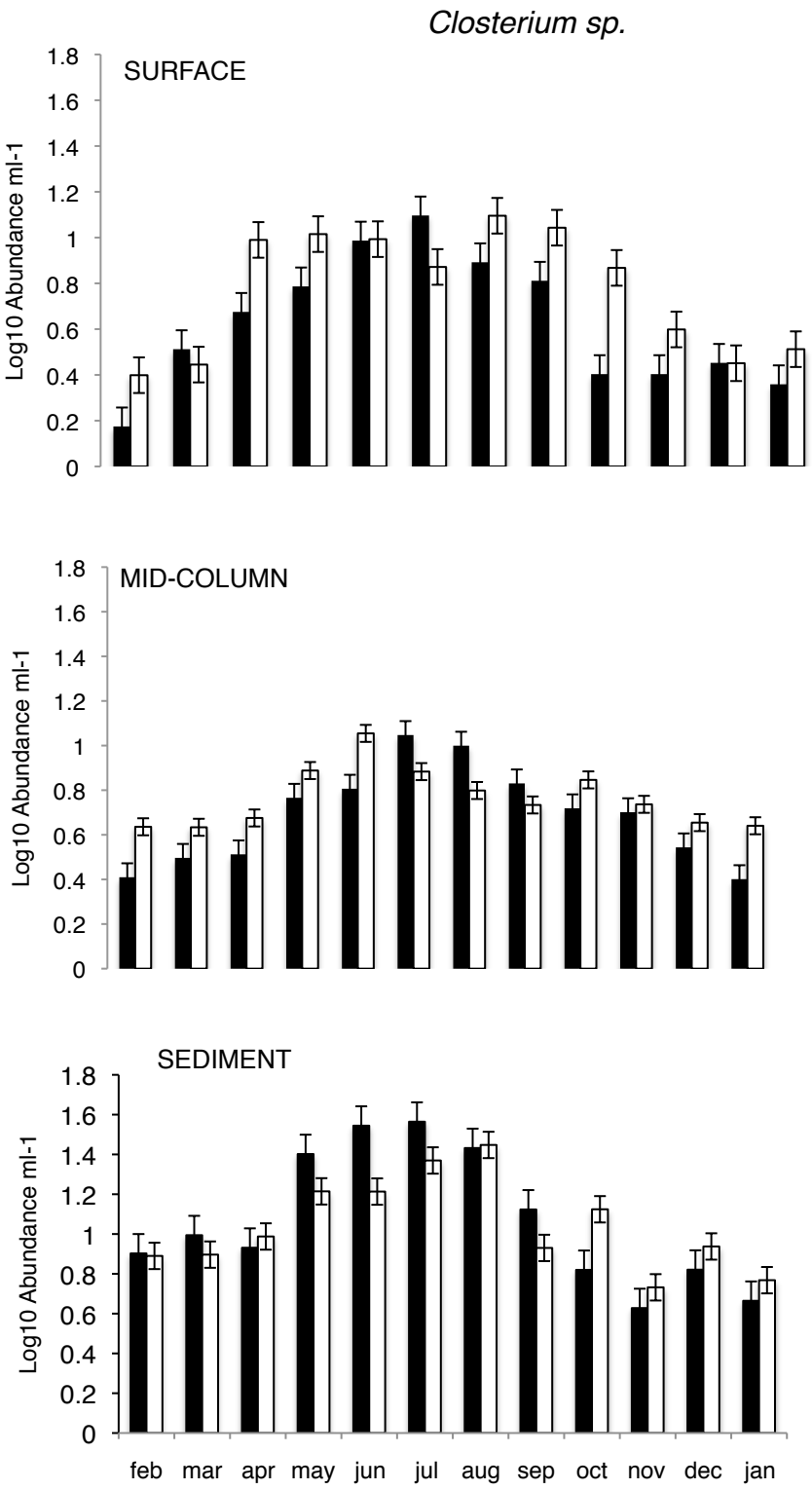


Figure 5.5 Log abundance of *Closterium spp.* over the sampling period February 2009-January 2010 in both warmed (white bars) and ambient (black bars) ponds. Error bars represent 1 standard error (±1SE).

Closterium sp. continued.

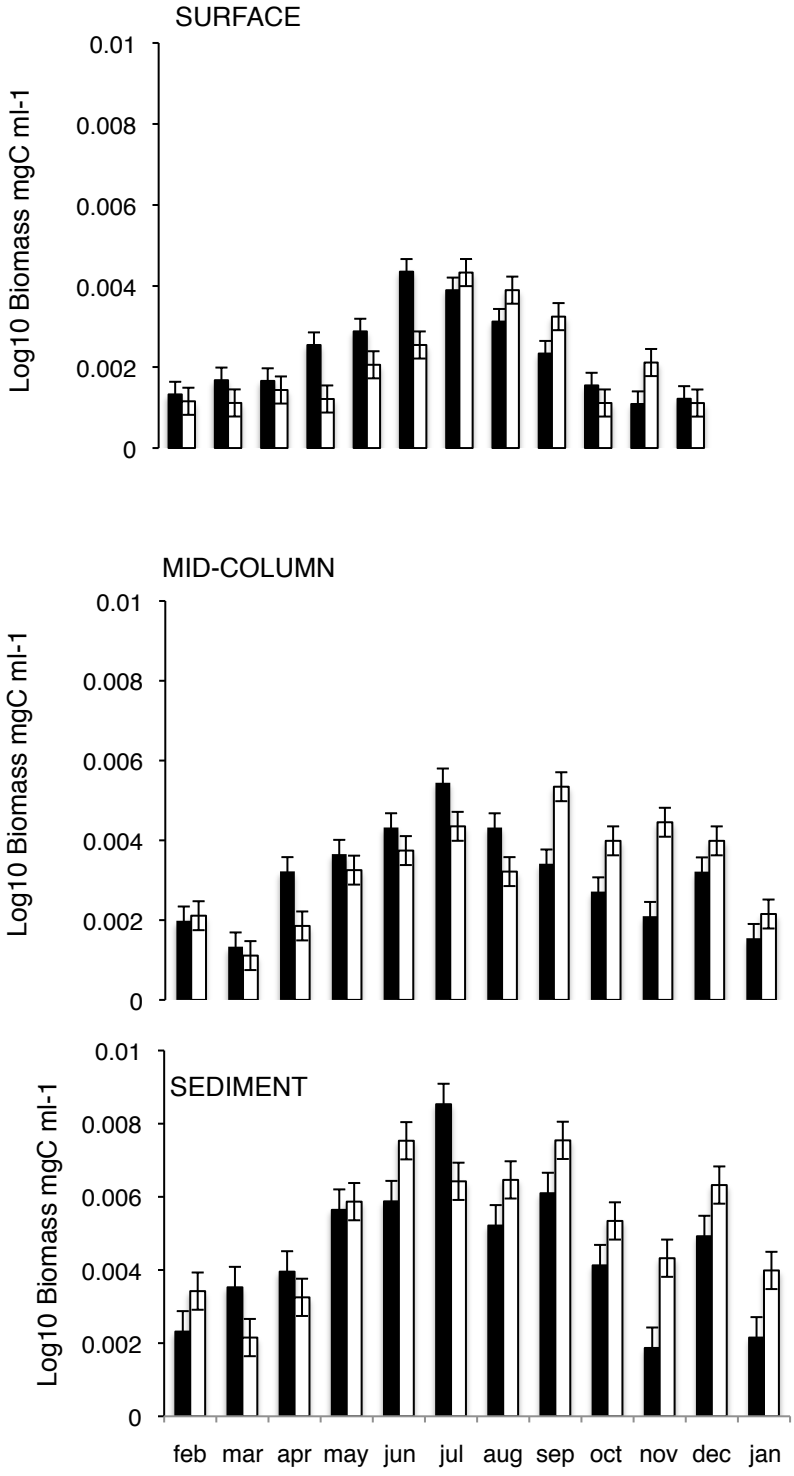


Figure 5.6 Log biomass (mgC ml-1) (±1 SE) for *Closterium sp.* over the sampling period February 2009-January 2010. Black bars represent ambient mesocosms, white bars represent warmed ones.

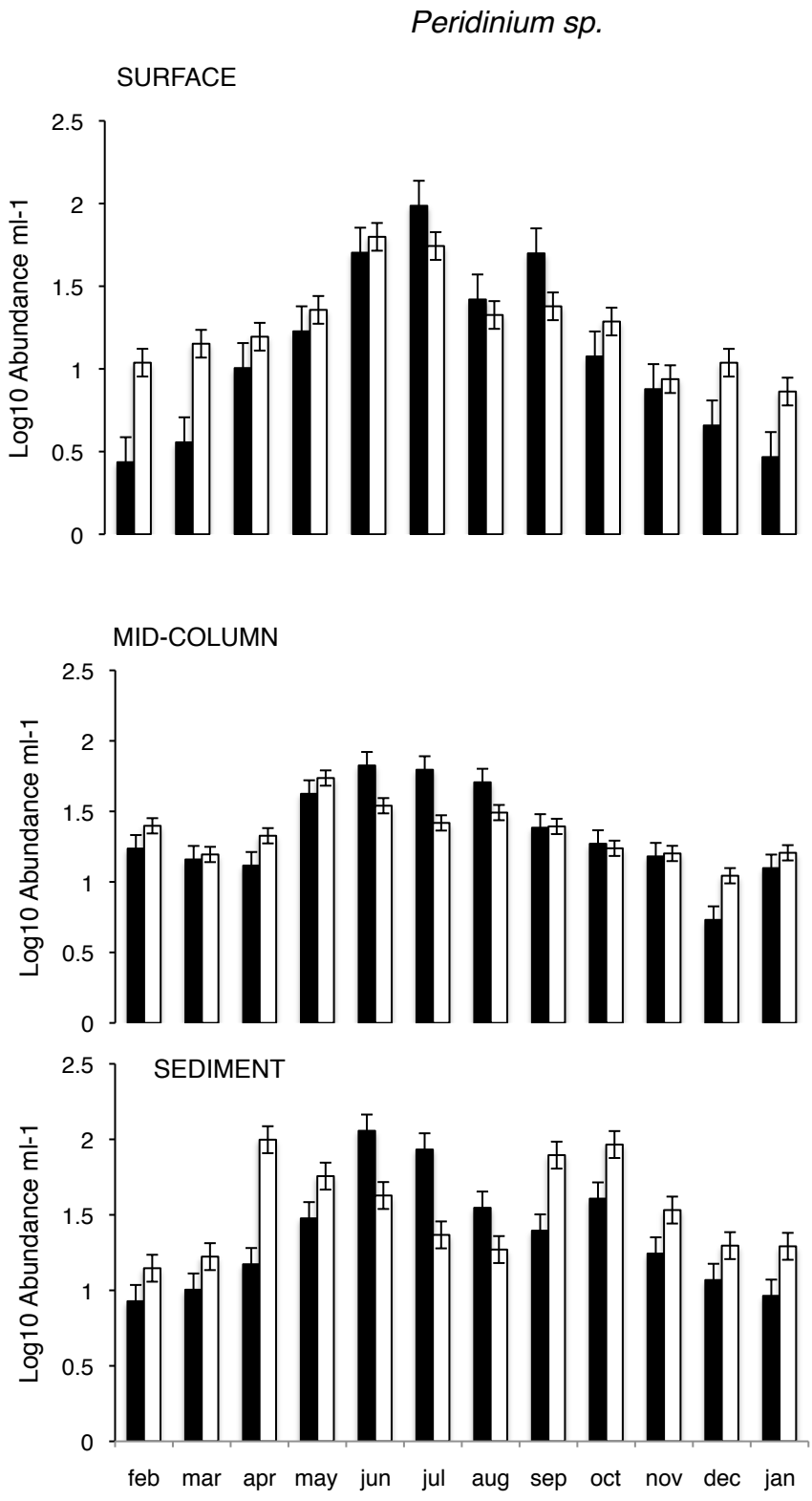


Figure 5.7 Log10 abundance of *Peridinium sp.* in the water column of the mesocosms (±1 SE) across the sampling period. Black bars represent ambient mesocosms, white bars represent warmed mesocosms.

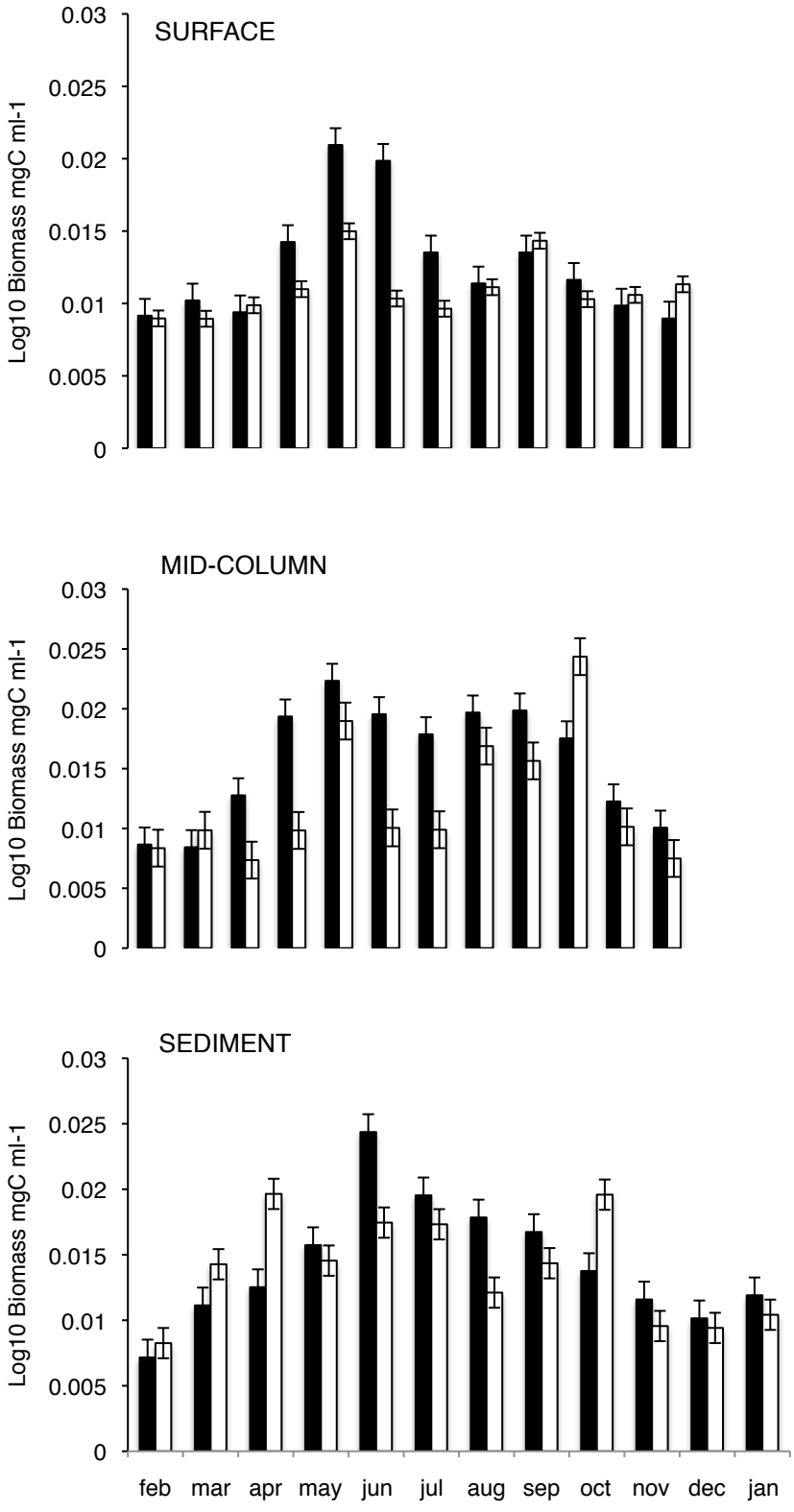


Figure 5.8 Population biomass (mgC ml^{-}1) of *Peridinium sp.* in the mesocosms across the sampling period (±1SE). Black bars represent ambient treatments, white represents warmed treatment.

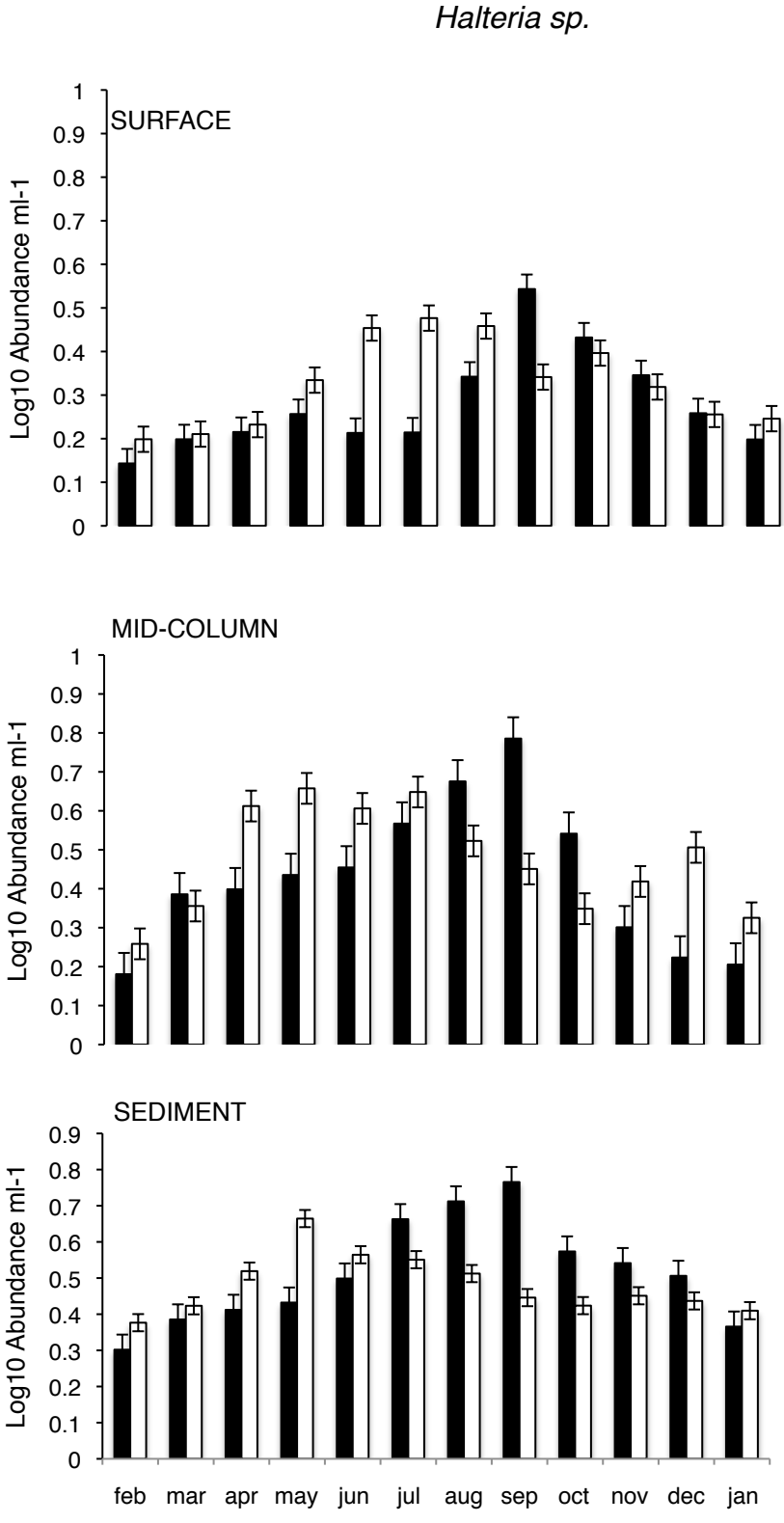


Figure 5.9 Log_{10} abundance (±1SE) of *Halteria sp.* in the warmed (white bars) and ambient (black bars) mesocosms, throughout the sampling period from February 2009 until January 2010.

Halteria sp. continued

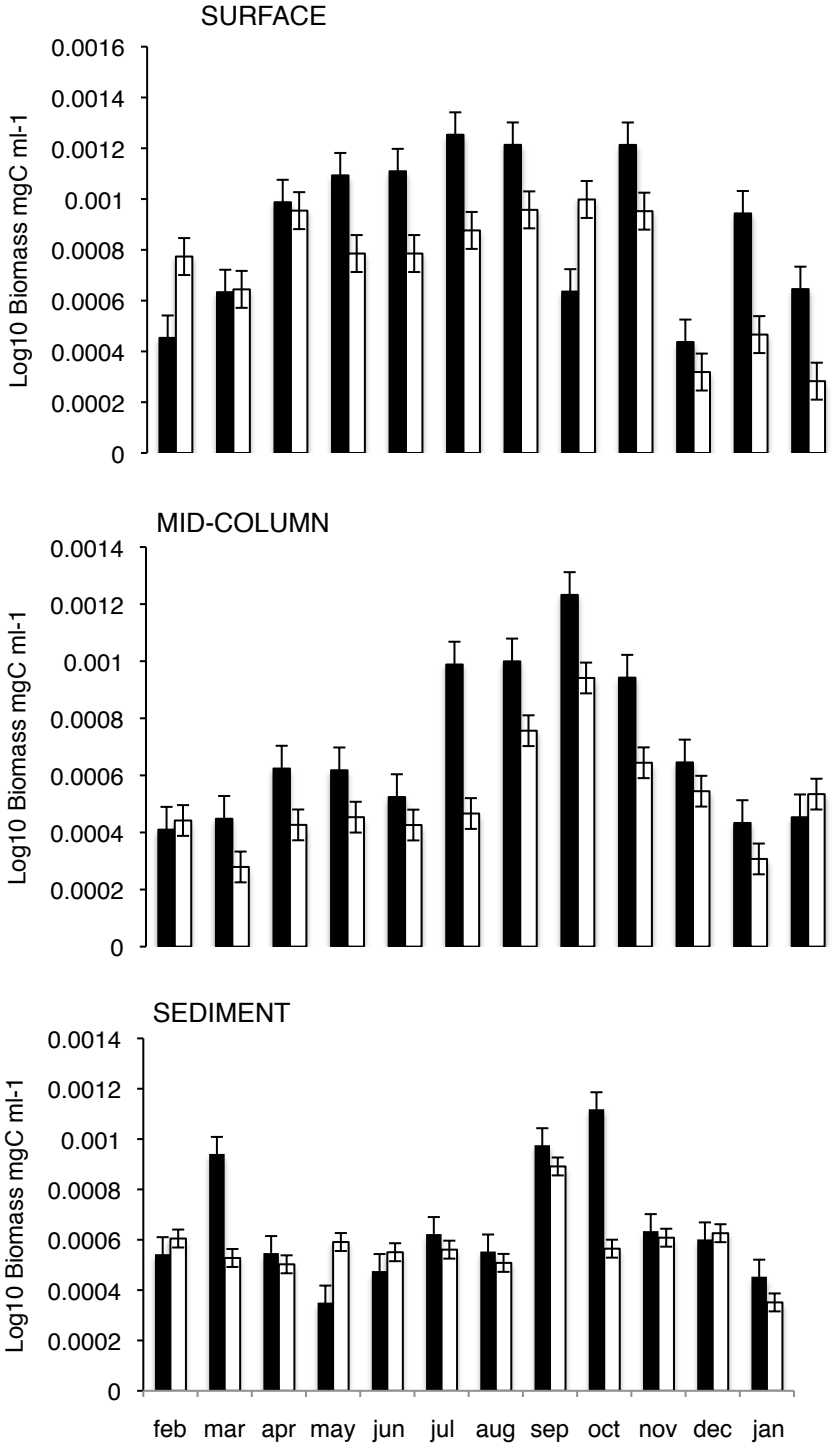


Figure 5.10 Log_{10} population biomass (mgC ml^{-1}) (±1SE) for *Halteria sp.* across the 12 month sampling period in warmed (white bars) and ambient (black bars) mesocosms.

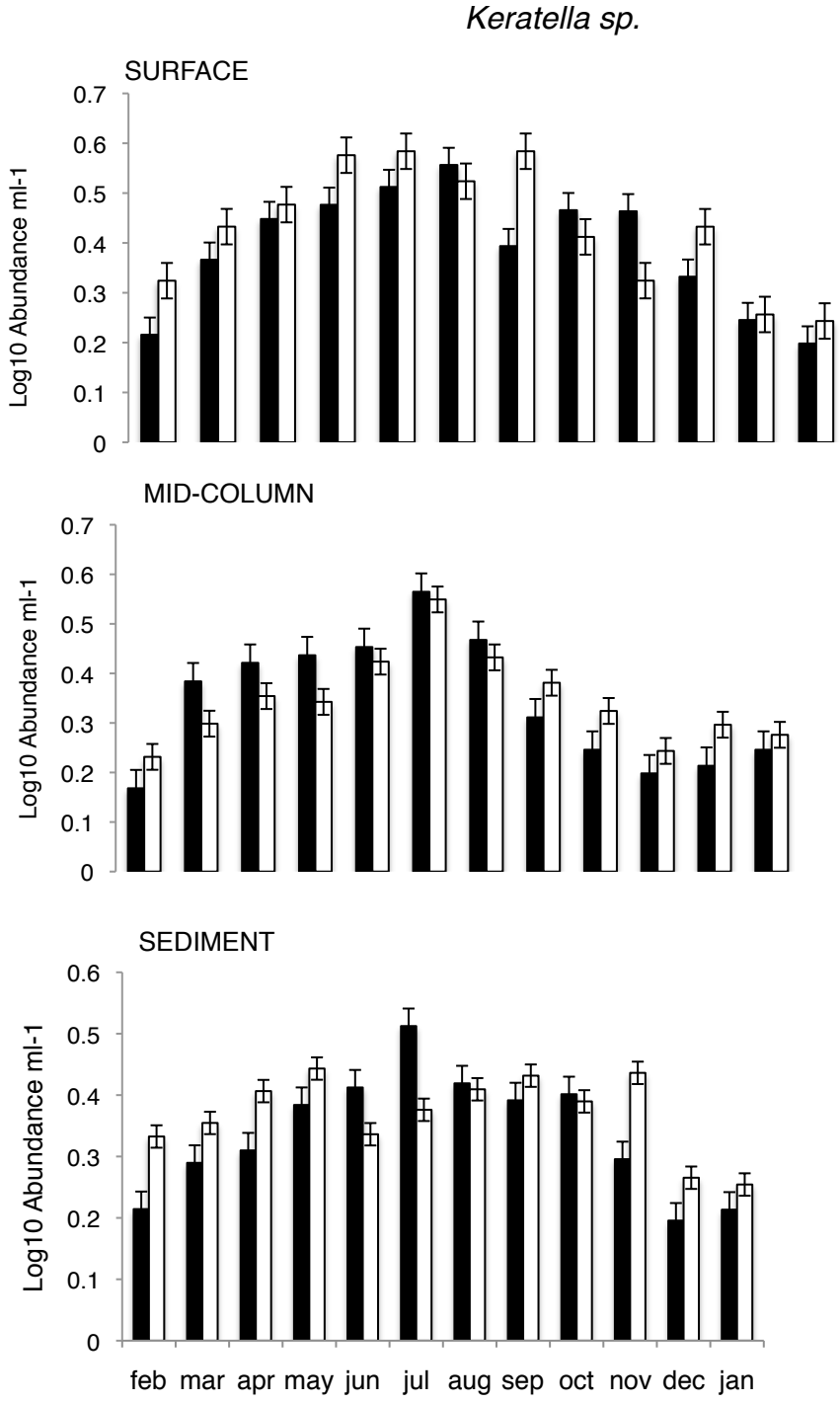


Figure 5.11 Log_{10} Abundance ml^{-1} of *Keratella sp.* over the 12-month sampling period in warmed (white bars) and ambient (black bars).

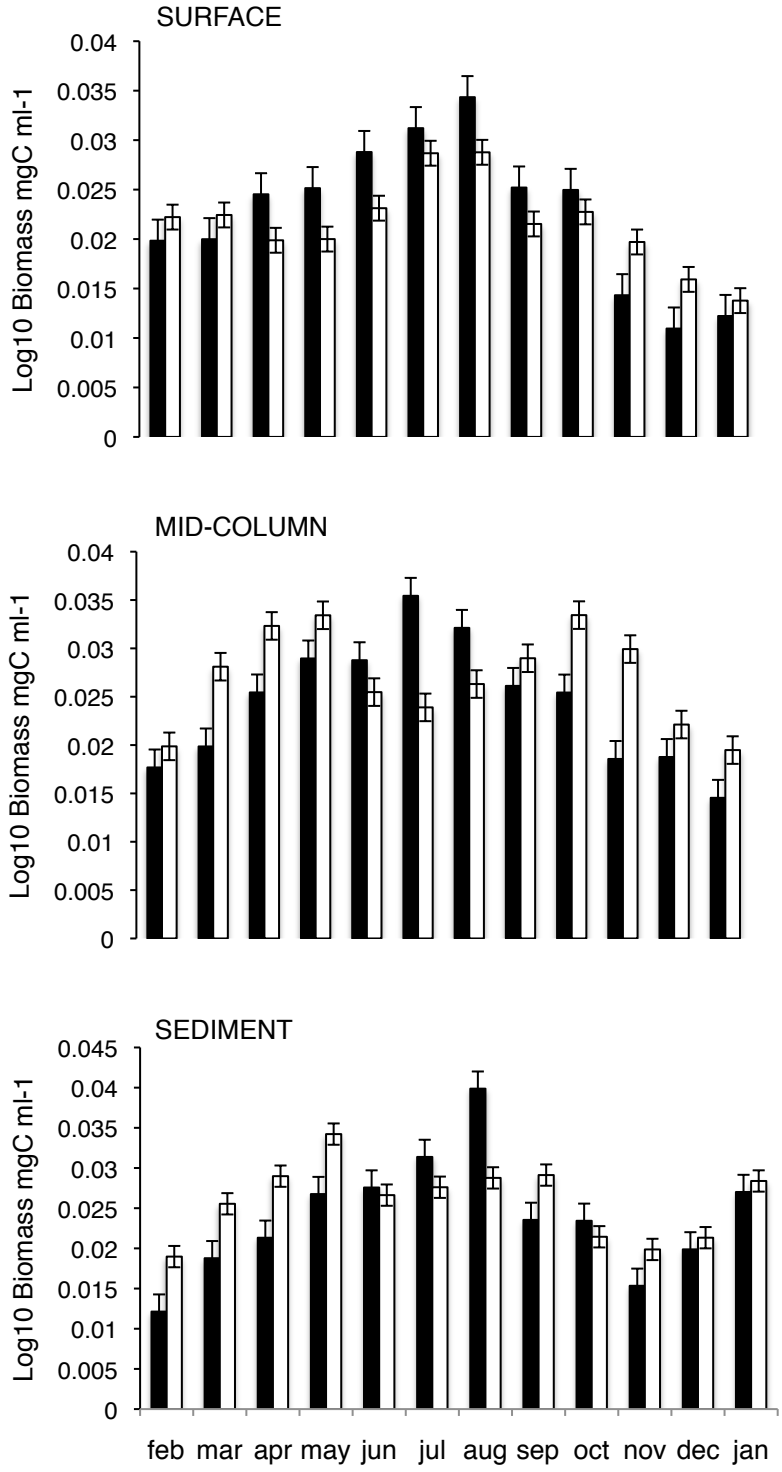


Figure 5.12 Log_{10} Biomass (μgC ml^{-1}) (±1SE) of *Keratella sp.* over the sampling period in warmed (white bars) and ambient (black bars) mesocosms.

5.5 Discussion

It is widely recognised that temperature has a major influence on the physiology of organisms because it controls basic metabolic processes. In particular, maximal growth rates of both autotrophic and heterotrophic organisms increase with temperature (Rose and Caron 2007). In this chapter, I investigated the effect of warming on the abundance, biomass and individual body mass of 4 populations of microbial loop organisms. I found there to be no overall main effect of warming on either individual body mass or abundance and biomass of the populations. However, there were significant two-way and three-way interactions with treatment and the spatial (depth) and temporal gradients. The implications of these interaction terms are far reaching in terms of effects at higher levels of organisation; there may be trophic decoupling in the natural food webs (Carrick *et al.* 1991; Winder and Schindler 2004). Mismatching is this manner has critical consequences at the ecosystem level, especially if keystone species are affected. For example, in aquatic ecosystems, algae-zooplankton interactions form the basis of energy flow from basal to higher trophic levels (Platt *et al.* 2003). Decoupling of this predator-prey relationship may be transmitted to all trophic levels, causing drastic ecological and economic consequences.

The interactive effect of temperature and the implications for shifts in food web structure have also been tested explicitly in microcosms (Petchey et al. 1999; Beveridge et al. 2010) and found significant effects of warming at these higher levels. The precise mechanisms behind such effects remain

unclear but are different for unicellular and multicellular organisms alike (see *chapter 1*)

Hypothesis (i): Individual body mass

Reduced body size in response to warming is a commonly observed phenomenon in ecology (Atkinson *et al.* 2003; Daufresne et al. 2009). With this phenomenon in consideration, *Hypothesis (i)* stated that warming would result in reduced overall body size in the genera tested. In 3 out of the 4 genera tested, warming appears to interact with the seasonal gradient (month) with a resultant significant effect on the body size of individuals during particular months. For example, *Closterium*, *Peridinium* and *Halteria* were seemingly unresponsive to temperature as a main effect in terms of body size reduction but exhibited significant responses to the interaction of temperature with the spatial gradient. This partially supports the TSR, with reduced body size seeming to be a taxon-specific and season-dependent effect (Walters and Hassall 2006). Despite extensive evidence for the existence of the TSR, no single general explanation exists (Angilletta and Dunham, 2004; Daufresne *et al.* 2009; de Jong, 2010; Forster *et al.* 2011). van der Have and de Jong (1996) proposed an explanation based on the unequal thermal sensitivities of growth (changes in mass) and development rate (changes in life stage) and showed that increased temperatures must cause a greater increase in development than in growth rates (Reiss *et al.* 2010). They suggest that their proposed mechanism is universal and thus applies to all organisms, including unicellular and multicellular organisms. Although the universality of their suggested mechanism has been questioned (Angilletta and Dunham 2004; Reiss *et al.*

2010), separating the effects of temperature into growth and development is a useful framework for developing ideas of the TSR (Kingsolver and Huey, 2008; Forster *et al.* 2011), and microscopic organisms offer particular promise in this regard because of their small size and short generation time for highly replicated warming experiments. However, some may prove to be more valuable in terms of extrapolating results and applying implications to higher taxa and processes, by using these types of studies in conjunction with surveys of natural systems and seeking common patterns. For example, parameters obtained from microcosms and mesocosms can be used to inform models which can then be tested in natural systems to assess how realistic responses in the laboratory may be when compared to responses in natural systems.

Hypothesis (ii) and (iii) Abundance and biomass

Warming did not have a significant main effect on the abundance and biomass of the 4 populations tested. In addition, the abundance and biomass of each population did not respond to warming in the same way for all genera e.g. *Peridinium* showed a significant response to warming in terms of abundance but not in biomass whereas *Halteria spp.* showed significant responses to warming in both abundance and biomass, indicating that again, there are species specific responses within major taxa of the microbial loop (*chapter 4*) and populations within populations of protists. This highlights the importance of furthering our understanding of the effect of warming on the smallest organisms in natural systems as they are not only ecologically and taxonomically diverse (Fenchel 1978), which may distort seemingly universal responses e.g in size

spectrum theory (*chapter 1*) but may also respond differently to warming, to different ends.

Caveats

The results here are for small organisms in mesocosms so caution should be applied, as always, when extrapolating and predicting future effects of warming to higher taxonomic groups and linking these results to the services provided by these organisms in natural systems.

Conclusion and future directions

I have shown that temperature has a powerful but subtle effect on population attributes (individual body size, abundance and biomass) when interacting with existing gradients (temporal and spatial), which has been evident at the community level (*in chapter 4*) and at the population level of some of the most dominant protists and meiofauna.

Future work should focus on more accurate ways of characterising populations than the classical methods of counting and measuring individuals of the microbial loop (e.g. in terms of abundance and functional groups of these important organisms for example, the increasing use of next generation sequencing and fingerprinting techniques in ecology will advance the speed and accuracy with which taxonomic and functional data can be obtained about these small organisms in natural systems [see *chapter 7* of this thesis (Purdy *et al.* 2010; Pilloni *et al.* 2012)].

5.6 References

Angilletta, M.J., and Dunham, A.E. (2004) The temperature-size rule in ectotherms: Simple evolutionary explanations may not be general *Am. Nat.* **162**, 332–342

Atkinson, D. (1994) Temperature and organism size—A biological law for ectotherms *Adv. Ecol. Res.* **25**, 158

Atkinson, D. (1995) Effects of temperature on the size of aquatic ectotherms: Exceptions to the general rule*Journal of Thermal Biology* **20**, 61-74

Atkinson, D., Ciotti, B.J., and Montagnes, D.J.S. (2003). Protists decrease in size linearly with temperature: Ca. 2.5% degrees C-1. *Proc. R. Soc. Lond. B Biol.* **270**, 2605–2611

Benton, T.G., Solan, M., Travis, J.M.J., and Sait, S.M. (2007) Microcosm experiments can inform global ecological problems*Trends Ecol. Evol.* **22**, 516–521

Bergmann, K.G.L.C. (1847) Über die Ver- hältnisse der wärmeokönomie der Thiere zu ihrer Grösse. *Göttinger Studien*, **3**, 595– 708

Beveridge, O.S., Humphries, S., and Petchey, O.L. (2010) The interacting effects of temperature and food chain length on trophic abundance and ecosystem function *J. Anim. Ecol.* **79**, 693–700

Brown, J.H., Gillooly, J.F., Allen, A.P., Savage, V.M., West, G.B. (2004) Toward a metabolic theory of ecology *Ecology* **85**: 1771–1789

Carpenter, S.R. (1996) Microcosm experiments have limited relevance for community and ecosystem ecology *Ecology* **77**, 677–680

Carrick H.J. and Fahnenstiel G.L. (1990) Planktonic protozoa in lakes Huron and Michigan – seasonal abundance and composition of ciliates and dinoflagellates. *Journal of Great Lakes Research*, **16**, 319–329

Carrick, H.J., Fahnenstiel, G.L., Stoermer, E.F. and Wetzel, R.G. (1991) The importance of zooplankton-protozoan trophic couplings in lake Michigan *Limnology and Oceanography*, 36, 1335–1345

Damuth, J. 1981 Population-density and body size in mammals. *Nature* **290**, 699-700

Damuth, J. (1987) Interspecific allometry of population density in mammals and other animals: The independence of body mass and population energy-use*Biol. J. Linn. Soc.* **31**, 193–246

Daufresne, M., Lengfellner, K., and Sommer, U. (2009) Global warming benefits the small in aquatic ecosystems*Proceedings of the National Academy of Sciences of the United States of America* **106**, 12788-12793

Fenchel, T. (2008) The microbial loop-25 years later*Journal of ExperimentalMarine Biology and Ecology,* **366,** 99-103

*Finlay, B.J and Esteban, G.F (1998) Freshwater protozoa: biodiversity and ecological function Biodiversity and Conservation **7,** 1163-1186*

Forster, J., Hirst, A. G., and Woodward, G. (2011) Growth and development rates have different thermal responses *American Naturalist* **178**, 668-678

Gasol, J.M., Guerrero, R., and Pedrosalio, C. (1991) Seasonal variations in size structure and prokaryotic dominance in sulphurous Lake Ciso *Limnol. Oceanogr.* **36**, 860–872

Hakenkamp, C.C., Morin, A. and Strayer, D.L. (2002) The functional importance of freshwater meiofauna. In: Freshwater Meiofauna: Biology and Ecology (Eds S.D. Rundle, A.L. Robertson and J.M. Schmid-Araya), pp. 321–335. *Backhuys Publishers, Leiden*

Hillebrand, H., Dürselen, C.-D., Kirschtel, D., Pollingher, U., and Zohary, T. (1999) Biovolume calculation for pelagic and benthic microalgae. *Journal of Phycology* **35**, 403-424

Jacobsen, D., Schultz, R. and Encalada, A. (1997) Structure and diversity of stream invertebrate assemblages: the influence of temperature with altitude and latitude *Freshwater Biology*, **38**: 247–261

James, F. C. (1970) Geographic Size Variation in Birds and Its Relationship to Climate *Ecology* **51**, 365-390

Kleiber, M. (1947) Body size and metabolic rate *Physiological Reviews***27**, 511–541

Parmesan, C. and Yohe, G. (2003) globally coherent fingerprint of climate change impacts across natural systems *Nature* **421**, 37-42

Parmesan, C. (2006) Ecological and evolutionary responses to recent climate change *Annual Review of Ecology Evolution and Systematics* **37,** 637-669

Peters, R.H. (1983) The ecological implications of body size *Cambridge University Press, Cambridge*

Petchey, O., Mcphearson, P., Casey, T.and Morin, P. (1999) Environmental warming alters food-web structure and ecosystem function *Nature* **402,** 69-72

Petchey, O.L. and Belgrano (2010) Body-size distributions and size-spectra: universal indicators of ecological status? *Biol. Lett.* doi: 10.1098/rsbl.2010.0240

Pilloni, G., Granitsiotis, M.S., Engel, M., Lueders, T. (2012) Testing the Limits of 454 Pyrotag Sequencing: Reproducibility, Quantitative Assessment and Comparison to T-RFLP Fingerprinting of Aquifer Microbes*PLoS ONE* **7**,7:e40467. doi:10.1371/journal.pone.0040467

Purdy, K.J., Hurd, P.J., Moya-Laraño, J., Trimmer, M., and Woodward, G. (2010).Systems biology for ecology.*Adv. Ecol. Res.* **43**, 87–149

R Development Core Team (2011) R: A language and environment for statistical computing*R Foundation for Statistical Computing, Vienna, Austria ISBN 3-900051-07-0*, URL http://www.R-project.org/

Reiss, J. and Schmid-Araya, J. M. (2008) Existing in plenty: abundance, biomass and diversity of ciliates and meiofauna in small streams*Freshwater Biology* **53**, 652 – 668

Reiss, J., Forster, J., Cassio, F., Pascoal, C., Stewart, R., Hirst, A.G. (2010) When Microscopic Organisms Inform General Ecological Theory Ed: Woodward, G.*Integrative Ecology: From Molecules to Ecosystems Book Series: Advances in Ecological Research* **43,** p. 45-85

Russell, B.D., Thompson, J.A.I., Falkenberg, L.J. and Connell, S.D. (2009), Synergistic effects of climate change and local stressors: CO2 and nutrient-driven change in sub-tidal rocky habitats *Global Change Biology*, **15**: 2153–2162

Sheridan, J.A. and Bickford, D. (2011) Shrinking body size as an ecological response to climate change *Nature Climate Change* **1**, 401-406

Sherr, E.B. and Sherr, B.F. (1994) Bacterivory and Herbivory: Key Roles of Phagotrophic Protists in Pelagic Food Webs *Microb. Ecol.* **28,** 223-235

Stead, T.K., Schmid-Araya J.M. and Hildrew, A.G. (2003) All creatures great and small: patterns in the stream benthos across a wide range of metazoan body size *Freshwater Biology* **48,** 532–547

van der Have, T. M., and de Jong, G. (1996) Adult size in ectotherms: temperature effects on growth and differentiation *Journal of Theoretical Biology* **183,** 329–340

Walther GR, *et al.* (2002) Ecological responses to recent climate change *Nature* **416:** 389 –395

West, G.B., Brown, J.H., Enquist, B.J., (1997) A general model for the origin of allometric scaling laws in biology *Science,* **276**, 122-126

Winder, M., Reuter, J.E. and S. Geoffrey Schladow, S.G. (2009) Lake warming favours small-sized planktonic diatom species *Proc. R. Soc. B.* **276**, 427-435

Yozzo, D.J. and Smith, D.E. (1995) Seasonality, abundance, and microhabitat distribution of meiofauna from a Chickahominy River, Virginia tidal freshwater marsh *Hydrobiologia* **310,**197-206

Yvon-Durocher, G., Allen, A.P., Montoya, J.M., Trimmer, M., and Woodward, G. (2010a) The temperature dependence of the carbon cycle in aquatic

ecosystems*in Ed.Woodward, G. Integrative Ecology: From Molecules to Ecosystems* pp. 267-313

Yvon-Durocher, G., Jones, J.I., Trimmer, M., Woodward, G., and Montoya, J.M. (2010b) Warming alters the metabolic balance of ecosystems*Philosophical Transactions of the Royal Society B-Biological Sciences* **365**, 2117-2126

Yvon-Durocher, G., Montoya, J.M., Trimmer, M., and Woodward, G. (2011) Warming alters the size spectrum and shifts the distribution of biomass in freshwater ecosystems *Global Change Biology* **17**, 1681-1694

Chapter 6

Temperature and mass dependence of population decline in laboratory protist assemblages at intergenerational scales

6.1 Abstract

Microscopic organisms are ideal candidates for identifying patterns related to unprecedented rates of global warming where the use of larger metazoans is constrained by time and space. Metabolic theory predicts that individual metabolism dictates rates at which organisms obtain, assimilate and expend energy and thus sets the rate of ecological processes at all levels of biological organisation (individuals to ecosystems). I investigated the relationship between temperature, mass and the rate of population decline (to extinction) in 3 protist populations and used the theoretical framework derived from the metabolic theory of ecology (MTE) to test whether this relationship is predictable. I tested the following predictions in the model system; (1) Rates of population decline will be faster at warmer temperatures due to elevated metabolic demands of individuals, following the ¾ power law as suggested by the MTE; (2) The temperature dependence of population decline of the cultures follows current MTE theoretical predictions with a temperature dependence in the range 0.6-0.7 eV for heterotrophic cell metabolism; (3) Organisms will decline in body mass under resource depletion and at a faster rate at warmer temperatures (following

a ¾ power law and (iv) the decline in mass will have a temperature dependence in the range 0.6-0.7eV, in accordance with Arrhenius regression models and the estimated activation energy for heterotrophic metabolism.

Results showed that (1) Populations declined faster at higher temperatures for all three species, with the largest species showing far weaker responses than was the case for the smaller species; (2) Temperature dependence for each individual population was outside of the range for biological rates, as specified in the literature *Blepharisma sp.* (slope=1.2, intercept =44.1,p=0.8), *Paramecium sp.*(slope=0.81, intercept =28.7,p=0.003)and for *Tetrahymena sp.*(slope=0.28, intercept =8.81,p=0.02). (3) All species declined in mass as resources depleted, throughout the course of the experiment and at a faster rate at higher temperatures and (4) the temperature dependence of the average rate of cell mass decline was outside the range suggested by the MTE [*Blepharisma sp.* (slope=0.17, intercept =5.1,p=0.05) *Paramecium sp.* (slope=0.35, intercept =6.7,p=0.08) and*Tetrahymena sp.* (slope=0.22, intercept =8.81, p=0.06)].

6.2 Introduction

The effects of global warming

Global surface temperatures have risen by ~0.74°C over the past 100 years and global average ambient temperatures and are predicted to rise by a further 3.0 -5.0 °C over the next century (Houghton 2001, 2005; IPCC 2007; see *chapter 1* of this thesis). Since temperature is a key determinant of biological process rates across multiple levels of organisation, this is likely to have profound effects on many ecosystem processes *via* population and community dynamics, species richness and abundance as well as the distribution of species traits (e.g. Walther *et al.* 2002; Parmesan 2006; Walther *et al.* 2010; Yvon-Durocher 2011; Dossena et al. 2012 and see *chapter 1* of this thesis).

There is an abundance of literature describing the effects of increased temperature on natural populations and the individuals within them. Documented impacts include range shifts and changes in phenology (Parmesan 2006) and, more recently, reduced body size has been coined as the "third universal response to warming" (Angilletta and Dunham 2004; Daufresne 2009). This phenomenon has been commonly observed in major taxonomic groups of ectotherms (Atkinson *et al.* 2003; Daufresne *et al.* 2009; Walther 2010; Sheridan and Bickford 2011). For example, Atkinson *et al.* (2003) document a reduction in the cell volume of protists with increased temperature, by testing species from terrestrial and aquatic environments. Specifically, they showed that for every 1 °C increase, cells reduce in volume by approximately 2.5% of that volume at 15 °C. The mechanisms behind this apparent shrinking remain unconfirmed yet several have been posited (see *chapter 1* of this thesis). In this thesis, I have referred to James's rule (*chapter 5*) and the Temperature Size

Rule (TSR) (*chapter 3*) and in this chapter, focus on using the general framework from the Metabolic Theory of Ecology (MTE) to explain some effects of temperature at the individual and population level. These theories are non mutually exclusive and all (or none) may act with the overall result of reduced body size.

Metabolic rate is “the most fundamental biological rate” and according to the MTE, sets the pace of resource uptake and allocation in individuals (*sensu* Brown *et al.* 2004). The MTE offers a general frameworkfor predicting rates and patterns at higher organisational levels, including population dynamics (e.g. population increase: Savage *et al.* 2004). If metabolism scales predictably with both the body mass of an individual and its ambient environmental temperature, there should be a clear link to the effects of environmental warming, which should elevate rates and also favour smaller organisms within and across species. There are often considerable difficulties in relating the growth rates (and extinction rates) of microbial organisms to those of higher organisms in both the field and in the laboratory, largely due to logistic constraints and the far longer generation times involved (e.g. Savage *et al.* 2004), which may therefore be circumvented by using small organisms (short generation times) in microcosms (high replication capabilities) and applying the MTE as a general theoretical and predictive framework (Gillooly 2001, 2002; Brown *et al.* 2004, Savage *et al.* 2004).

The effects of temperature and body mass on metabolic rate and the combined effects of these and body size, is described by equation 1 for an individual (Gillooly *et al.* 2001). Nevertheless, it provides a useful heuristic

framework to investigate the temperature dependence of population dynamics in the laboratory setting and using pure cultures of common protists.

Equation 1 $$B(m,T) = B_0 e^{-E/kT} M^{3/4}$$

Where E is the temperature dependence of rate-limiting biochemical reactions and *k* is the Boltzmann constant that describes the effect of temperature on biochemical reactions. *B* is the basal metabolic rate of an individual, *m* is the mass of an individual; *T* is the temperature (Kelvin). The quarter power scaling used here, is related to the rate of resource supply through a fractal branching network, is explained in detail in Gillooly *et al.* (2001, 2002), but can also be used to relate to the rate of population decline in this laboratory study. The relationship between temperature, mass and population growth rate is described by equation 2.

Equation 2 $$M \text{ ¼ } e^{-E/kT}$$

Savage *et al.* (2004) linked population growth to metabolism using a combination of the equations 1 and 2 describing Malthusian population growth and equations that describe the temperature dependence of r_{max} in eukaryotic anaerobes at the individual and population leveland found that the rate of population increase was predictable in terms of the equations of metabolic theory across a wide range of ectothermic groups. In this chapter, I attempt to link metabolism, body size and population decline (to extinction) using three species of protists in laboratory microcosms as a mirror image of the findings from the meta-analysis by Savage *et al.* (2004). The species used were of the

same genera as those identified from the mesocosm experiment described in chapters 3-5, to allow further discussion of the results in both the microcosm and mesocosm setting.

The implications of shrinking body sizes in a warmer world are far reaching; firstly, in terms of the microbial species and their role in aquatic food webs is not well described or understood but it is known that these organisms play an important role in ecosystems in terms of carbon assimilation and energy transfer to higher trophic levels (Pomeroy 1974). It may be that the 'shrinking patterns' exhibited by small organisms used in model laboratory systems may reflect the impacts of warming on organisms in natural systems and aid the understanding of why reduced body size is a common response to warming and aid understanding in terms of mitigation of the effects of warming (Sheridan and Bickford 2011). Large and rapid changes to the size structure (as a result of smaller individual body sizes) at basal levels may lead to instabilities in population dynamic interactions within aquatic food webs as a whole (Angiletta and Dunham 2004; Daufresne *et al.* 2009; Sheridan and Bickford 2011; Dossena *et al.* 2012). In particular, changes in the phenology as a result may (see *chapter 5*)

Increasingly, microcosms and mesocosms, have been used to aid the prediction of the effects of warming on individuals, food webs and entire ecosystems underIPCC (2007) anticipated warming scenarios (e.g microcosms: Delaney *et al.* 2003; Newsham and Garstecki 2007; Beveridge *et al.* 2010; mesocosms: Baulch *et al.* 2005; Yvon-Durocher *et al.* 2011; Dossena *et al.* 2012). In this study, the use ofmicrocosms allowed me to observe of the effects

of simulated environmental warming on the rates of population decline on protists of different body masses, within a relatively short absolute time frame (8 weeks) and across hundreds of generations of protists. (e.g. Petchey *et al.* 1999; Delaney *et al.* 2003; Jiang and Morin 2004). The usefulness of microcosms for testing general ecological theory has been demonstrated in a range of studies to date [(e.g. Lawler and Morin 1993, Morin1999, Petchey *et al.*1999, Buckling *et al.* 2000, Benton *et al.* 2007; for a review: Reiss *et al.* 2010) see *chapter 1* of this thesis]. In addition, it is not always possible to obtain such accurate measures (due to complete control and high replication ability when using microcosm systems) for larger and slower-growing organisms, especially in natural systems, due to spatial and temporal constraints on much shorter generational scales (e.g. Jacobsen *et al.* 1997). I have outlined the advantages and disadvantages of different experimental approaches in *chapter 1*.

As well as the use of microcosms and mesocosms to measure the responses of microscopic animals in microcosms and mesocosms, the relationships between biological rates [(e.g development and growth rates (Forster *et al.* 2011)] have been mathematically modeled in different ways; for example, within-species, models based on linear (Montagnes *et al.* 2003), allometric (Belehradek 1926; McLaren 1969; Corkett and McLaren 1970; Hart 1990; Peterson 2001), and exponential (Escribano and McLaren 1992; Escribano *et al.* 1997; Campbell *et al.* 2001) functions have been applied to describe how development rates (in the examples above) change with temperature. More complex relationships with a mechanistic basis—for example, Arrhenius (Gillooly *et al.* 2002; Brown *et al.* 2004) have been used to a large extent and yielded a lot of research in the last 10 years, working to

either prove or disprove the MTE predictions, particularly in relation to the value of the precise mass-scaling exponents (multiples of 0.25) that are predicted and the underlying mechanism.

In another study examining the effect of warming on extinction, Allen *et al.* (2002) used the energetic equivalence rule (Damuth 1981, 1987) to predict that mass-corrected population abundance declines with warming in direct proportion to the temperature dependence of heterotrophic metabolism, assuming that the total energy flux of a population per unit area is invariant with respect to body size. As yet, no studies have linked individual mass and environmental temperature to population extinction rates explicitly, by testing the relationship in a controlled laboratory setting and in this chapter, I attempt to address this research gap using laboratory microcosms and 3 axenic populations of ciliates and test the ¾ power law suggested by the MTE. I tested the following hypotheses:

(i) If there is a significant relationship between temperature and rates of population decline then, at higher temperatures then population decline will be accelerated for all species.

(ii) Population decline to extinction will be fastest for the largest species if there a significant relationship between individual body mass, temperature and rate of population decline.

(iii) If population decline (under resource depletion) is exclusively attributable tothe metabolism of individuals (Brown *et al.* 2004; Savage *et al.* 2004) then the relationshipbetween the inverse of absolute temperature and the mass-corrected rate of decline (tested via the Arrhenius equations)

should yield a slope in the range of -0.6 and -0.7 for heterotrophic metabolism (Gillooly *et al.* 2001,2002).

(iv) There will be a measurable decline in cell volume as population density declines, due to resource resource depletion (DeLong *et al.* 2009) and this loss should occur more rapidly at higher temperatures due to elevated metabolic demands (Clarke and Fraser 2004).

6.3 Methods

I quantified the rate of population decline in three aquatic, unicellular protist species (ciliates), across an experimentally manipulated but realistic, biologically meaningful temperature range (10-25 °C) that these organisms are exposed to in natural aquatic systems (e.g. in freshwater ponds), to characterise the potential impacts of warming in aquatic systems. I then sought to integrate three key variables (body mass, temperature and population density) to test the application of the temperature size rule (by measuring the body size of individuals) and the MTE to the rate of population decline (after Brown *et al.* 2004).

Experimental design and set-up

The experiment was carried out in laboratory microcosms at the university of Sheffield in May until July 2010 that consisted of 250ml jars containing 100ml of culture media, which were fitted with a foil lid to allow gas exchange whilst preventing contamination (Beveridge *et al.* 2010). The culture medium was Chalkley's (Tompkins *et al.* 1995) with 0.55 g/l "protist pellet", which provided a source of organic nutrients (Carolina T.M. Protozoan pellets, Burlington, NC,

USA). The Chalkley's medium, containing the crushed pellet was added to one litre of water and then autoclaved. Standard aseptic techniques were used in the laboratory; all pipettes were autoclaved before use and the microcosms remained covered at all times (see Burlage *et al.* 1998 and *chapter 2* of this thesis). Each microcosm was inoculated with 30ml of a pure culture of one of the 3 ciliate species.

The following three species were used; an omnivorous heterotrich, *Blepharisma japonicum* (Linn.), and two oligohymenophoran ciliates, *Tetrahymena pyriformis* (Ehr.) and *Paramecium caudatum* (Ehr.). These were chosen because they are easy to cultivate in the laboratory and are fast growing, versatile species, tolerant of a wide range of temperatures (Asai *et al.*2009) (and were readily available in existing laboratory cultures). The 3 pure cultures ('axenic') ciliate populations were grown from existing laboratory stock solutions (Carolina T.M. Protozoan cultures, Burlington, NC, USA), in four 2-litre flasks per species. These flasks were maintained at 20 °C in a controlled-temperature room until each population reached carrying capacity, *K,* defined when population growth curves plots reached a plateau (see Figure 6.1).

Population growth curves were plotted for each species (Figure 6.1) by regular estimation of species density within the flasks. Population densities were estimated every 2 days by the removal of small, 0.25ml aliquots (i.e., entire microcosms were not removed from the treatments), and densities were estimated *via* direct counting under a dissection microscope (Nikon SMZ1000) and only active, motile (as opposed to moribund) cells were counted.

Once each population reached carrying capacity in the 2-litre flasks, cultures were agitated to homogenise the populations, and then 100ml of each

culture was subsequently removed and transferred into a set of 250 ml jars (i.e. replicate microcosms). The microcosms were then each subjected to one of the 7 experimental temperature treatments, which were controlled at approximately 3-5 °C intervals along a thermal gradient (10, 15, 20, 16.5, 18, 22.5 and 25 °C). I then derived the rate of each population decline from the density estimates every 2 days for the 8-week duration of the experiment.

The experimental design was fully factorial, with each treatment replicated 4 times for *Blepharisma japonicum* and *Tetrahymena hymena* and three times for *Paramecium caudatum*: thus 84 microcosms were used in total (i.e. 3 species x 4 (or 3) replicates x 7 temperature treatments). Finally, I obtained estimates for the temperature dependence and mass dependence of population decline for individual populations and the combined rates of all, and compared the values obtained for population decline with those from existing studies of other biological rates e.g. rate of population increase by Savage *et al.* (2004).

Body size estimates

To obtain length and width measurements, a sample of each ciliate population was placed on a haemocytometer and a 35 second video was recorded, through a stereomicroscope, using a digital camera. Using the haemocytometer to establish an absolute scale, cell dimensions (length and width) Were measured digitally using Image J, image processing and analysis software in Java (Abramoff *et al.* 2004). Cell volume and individual biomass was calculated following methods outlined in Wetzel and Likens (1991) and Hillebrand *et al.*

(1999), using the linear measurements from an average of at least 10 individuals per replicate.

Statistical analysis

The rate of population decline (cells in the known volume of medium day^{-1}) was calculated from population density (cells ml^{-1}) estimates, as population density declined from *K*. All results were analysed using R software, version 2.9.1 (R Development Core Team 2007).

Polynomial regression models were first fitted to the data using ordinary least squares regression because they are a useful means of exploring more complex concepts and describe the linearity of data. The polynomial models confirmed that the data collected are non-linear (the best fitting models are summarised in tables 6.1 and 6.2).

To test the framework of the MTE, species rates were plotted against the inverse of temperature in Kelvin (Figures 6. and I fitted the several models including (*Table 6.1*) Arrhenius regression models of population decline and rate of body size reduction to the species data from our microcosms. I compared the fit of each model using the Akaike Information Criterion (AIC) (Akaike 1974). The models tested are summarised in Table 6.1. In addition to using the MTE framework to examine the rate of population decline, I used the models from the literature that describe growth and development and applied them to the rates of population decline for the 3 protistpopulations.

Table 6.1 Adapted from Forster *et al.* (2011); different models used in examining biological rates. I have applied these models to the population decline data to test whether the Arrhenius model best describes the data using AIC methods of model selection.

Model	Equation	Statistical Model	Reference
Allometric	$R= aT^b$ + error	lnR=lna+blnT+error	Belehradek 1926
Complex Allometric	$R=aT(^{b+clogT)}$+error	$lnR=lna+blnT+c(lnT)^2$+error	O'Connor et al. 2007
Exponential	$R=ae^{bT}$+ error	lnR=lna+bT+error	Campbell et al. 2001
Arrhenius	$R=ae^{-Ea/kT(K)}$+error	$lnR=lna-b(1/kT_{(K)})$ + error	Cossins and Bowler 1987

R p rate (day 1, growth or development); a, b, and c are constants; T is temperature (°C); T (K) is temperature (degrees Kelvin); k is Boltzmann's constant (8.617 x 10^5eV K^{-1}); and $E_a$is average activation energy for the rate-limiting enzyme-catalyzed biochemical reactions of metabolism (c.f. Brown *et al.* 2004)

6.4 Results

Hypothesis (i): Rate of population decline

Temperature significantly increased the rate of population decline under resource depletion for all three species and all three species went completely extinct at 22.5 °C and 25 °C, within the experimental period of 40 days (see Figure 6.2). At the lowest temperatures (10 °C and 15 °C), all the populations declined markedly but did not go extinct and viable counts were still made throughout and after the 6-week experimental period (Figure 6.1).

The rate of population density decline also differs with body size. Firstly, the largest species (*Blepharisma*) has a less pronounced "base" rate of decline (i.e. decline at a middle temperature of 15 °C) than *Paramecium*, which has a less pronounced "base" rate of decline than *Tetrahymena* (Table 6.1). The change in rate of decline from "base" rate to the higher temperatures in *Blepharisma* is from approx. 0 to -0.08 cells day^{-1}, in *Paramecium* is approx. -0.025 to -0.07cells day^{-1} and in *Tetrahymena*is from approx -0.06 to -0.1cells

day^{-1}. This suggests that the smallest species shows the least change in rate of decline in numbers with temperature, and the largest species shows the greatest change in rate of decline, i.e. population decline of smaller species are less affected by temperature than larger species, as predicted by hypothesis (ii) (Figure 6.2).

Hypothesis (ii): Temperature dependence of population decline

The average temperature dependence of population decline differed among species, although the average value across all three fell outside [see Table 6.1(although close to the upper limit) of the range specified in Gillooly *et al.* 2001 (0.6-0.7 eV) (slope of Arrhenius plots of -0.71eV;r^2=0.263, $F_{1,19}$ =8.141, p=0.0102). This lends support to hypothesis (ii) that population decline to extinction will be fastest for the largest species if there a significant relationship between individual body mass, temperature and rate of population decline.

Hypotheses (iii) and (iv): Changes in body mass

Results showed that individuals also became smaller over time, in agreement with hypothesis (iii). The average individual body mass of all three species declined over time, under resource depletion (Figure 6.4) and the rate of body mass decline versus temperature is comparable across species (Table 6.2), despite the greater response of the largest ciliate to warming in terms of population density. The temperature dependence of the rates of cell shrinking (Figure 6.5) is lowest for the largest species and highest for the intermediate species (Table 6.2). Which supports the quantitative predictions of the MTE.

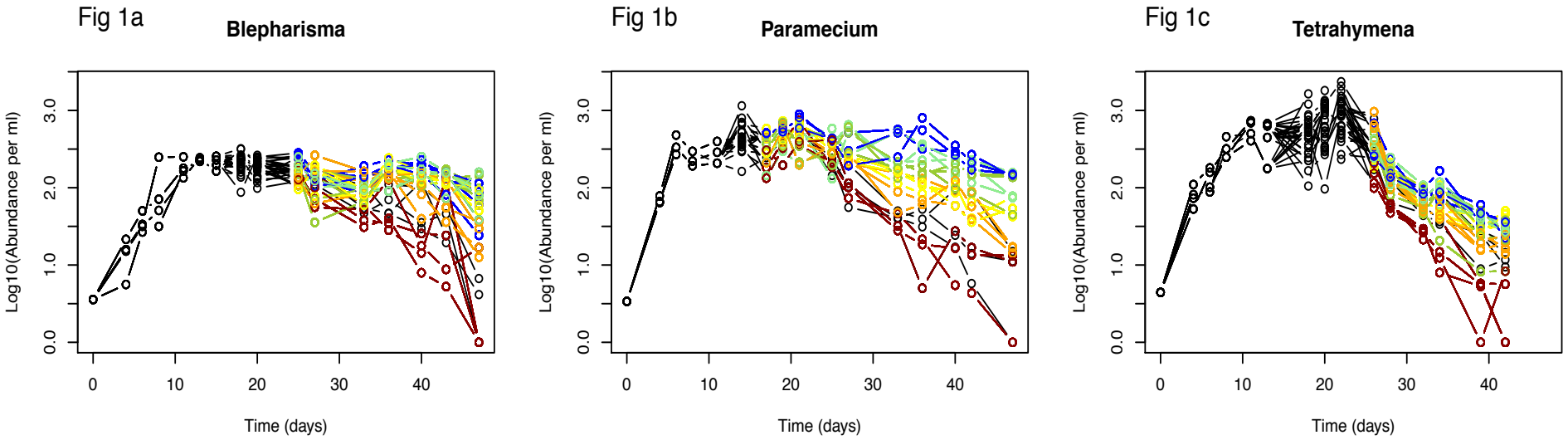


Figure 6.1 Population curves for the three populations of protist. Black lines show exponential growth phase at 20 °C, in 2-litre flasks. Coloured lines indicate when the protists were put under temperature treatment at a range of temperatures in 250-ml microcosms.

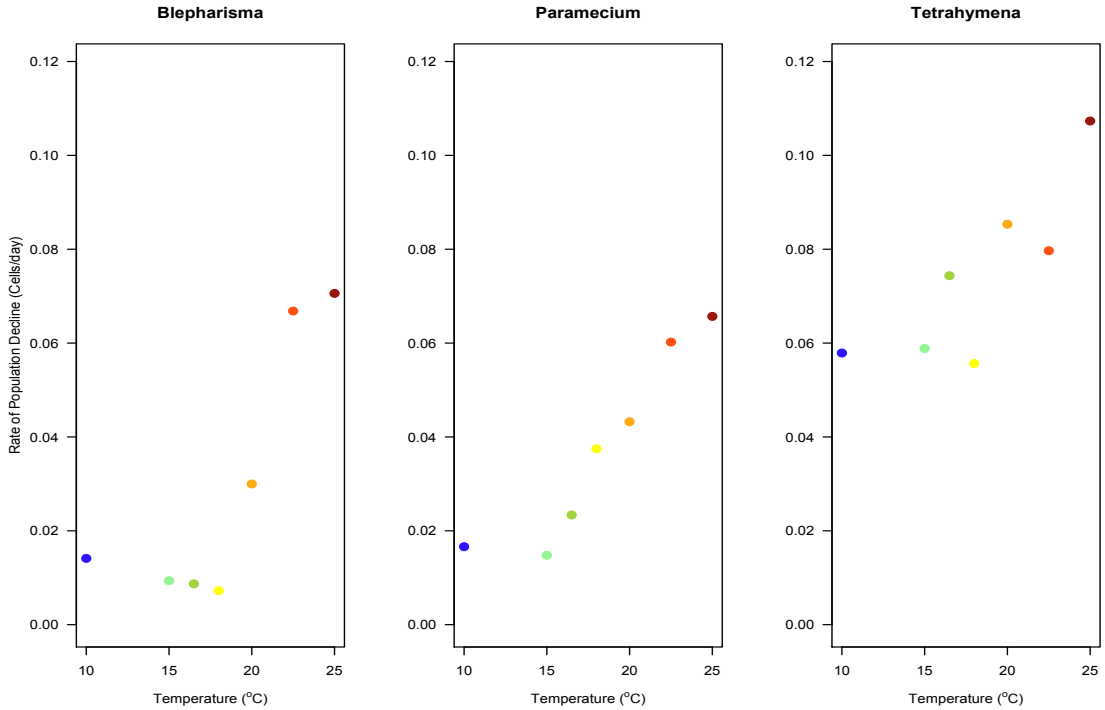


Figure 6.2 Rates of population decline by species. Coloured points correspond to the range of temperatures the populations were subjected to after carrying capacity was reached and correspond to the colour scheme used in the population growth curves (Figure 6.1).

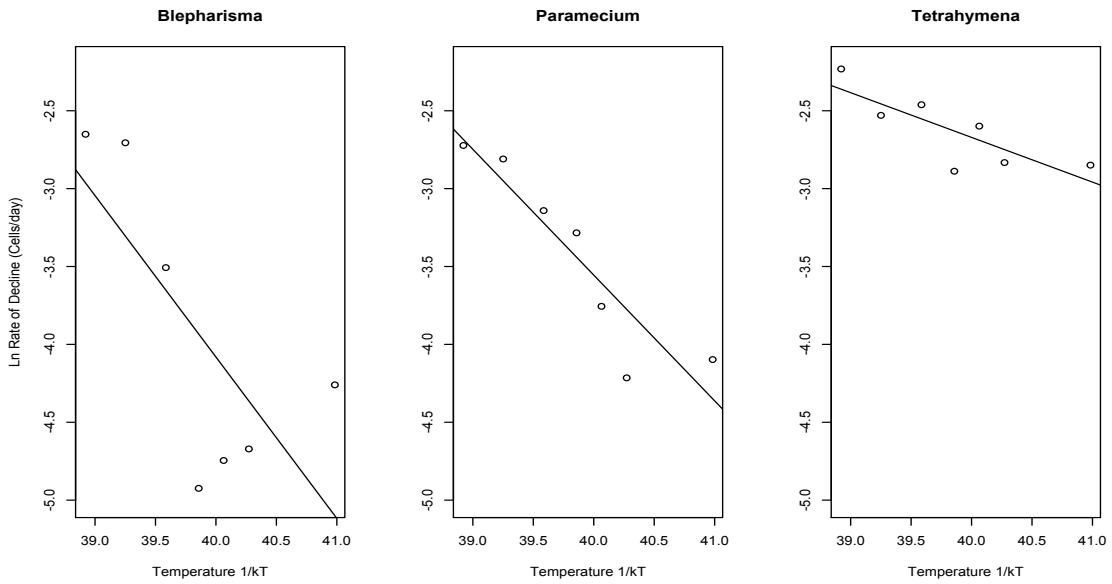


Figure 6.3 Arrhenius plots for each species, describing the temperature dependence of the rate of population decline. The temperature dependencies differ significantly between species (*Table 6.2*)

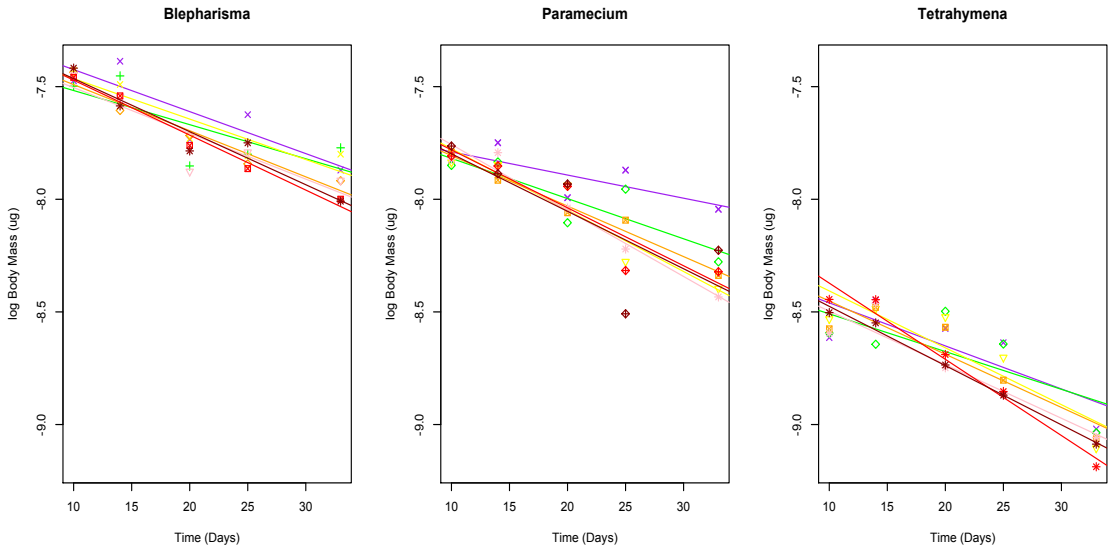


Figure 6.4 The natural log of average individual body mass over time. The slopes indicate the rate of body mass decline. Different coloured points and lines correspond to different temperatures at which the populations were subjected to (purple = 10°C, green = 15°C, yellow =16.5 °C, orange =18 °C, pink=20°C bright red= 22.5°C, dark red= 25°C)

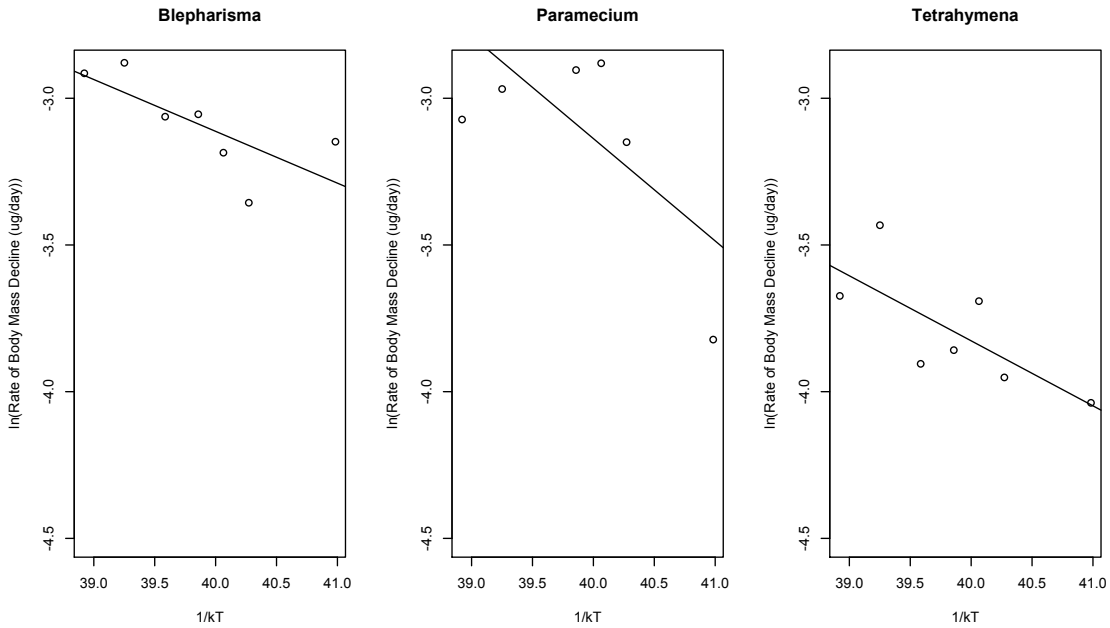


Figure 6.5 Arrhenius plots for each species, describing the temperature dependence of the rate of cell shrinkage. The temperature dependencies differ significantly between species (see *Table 6.3*).

Table 6.2 Best fitting polynomial regression models for population decline rates for each population, listed smallest to largest. Rates of population decline at 15 °C (mid-temperature) in comparison to rate of decline at 20 °C. Temperature dependence refers to individual slopes of Arrhenius plots for individual species (rate of decline versus the inverse temperature).

Size	**Species**	**Decline at 15 °C (Cells day^{-1})**	**Decline at 20 °C (Cells day^{-1})**	**Phenomological model (best fit)**	**Temperature dependence**
Smallest	*Tetrahymena pyrifromis*	0.0589	0.0853	2nd order polynomial (R^2 =0.61, DF = 1,6,F =8.95, p=0.0177)	Slope=0.28, intercept =8.81, p=0.02
	Paramecium caudatum	0.0148	0.0432	3rd order polynomial (R^2 = 0.99, DF = 3,3, F=82.96, p=0.00216)	Slope=0.81,intercept =28.7, p=0.003
Largest	*Blepharisma japonicum*	0.00936	0.04	2nd order polynomial R^2 = 0.85, DF =2,5 F= 13.72, p=0.00933	Slope=1.21, intercept=44.1,p=0.8

Table 6.3 Average rates of individual cell shrinkage from the start of warming treatment and best fitting models listed in order of initial body size from smallest to largest. Temperature dependence refers to the slope and intercept of individual species shrinkage rates plotted against the inverse of temperature.

Size	Species	Shrinkage rate 15 °C ($\mu g\ day^{-1}$)	Shrinkage rate 20 °C ($\mu g\ day^{-1}$)	Linear Regression	Temperature dependence
Smallest	*Tetrahymena pyriformis*	$4.94x10^{-5}$	$1.33x10^{-3}$	R^2=0.65, DF=6, F= 8.95, p<0.029	Average slope=0.22, intercept=5.03, p=0.06
	Paramecium caudatum	$6.44x10^{-3}$	$3.55x10^{-3}$	R^2=0.65, DF=6, F= 14.95, p<0.001	Average slope= 0.35, intercept =10.82,p=0.08
Largest	*Blepharisma japonicum*	$1.14x10^{-2}$	$8.9x10^{-2}$	R^2=0.65, DF=6, F= 10.34, p<0.022	Average slope=0.17, intercept = 3.95, p=0.05

Combining all rates

To further test the MTE framework [(hypothesis (ii)], using the population decline rate, the combined rates were plotted against the inverse of temperature (Figure 6.6)

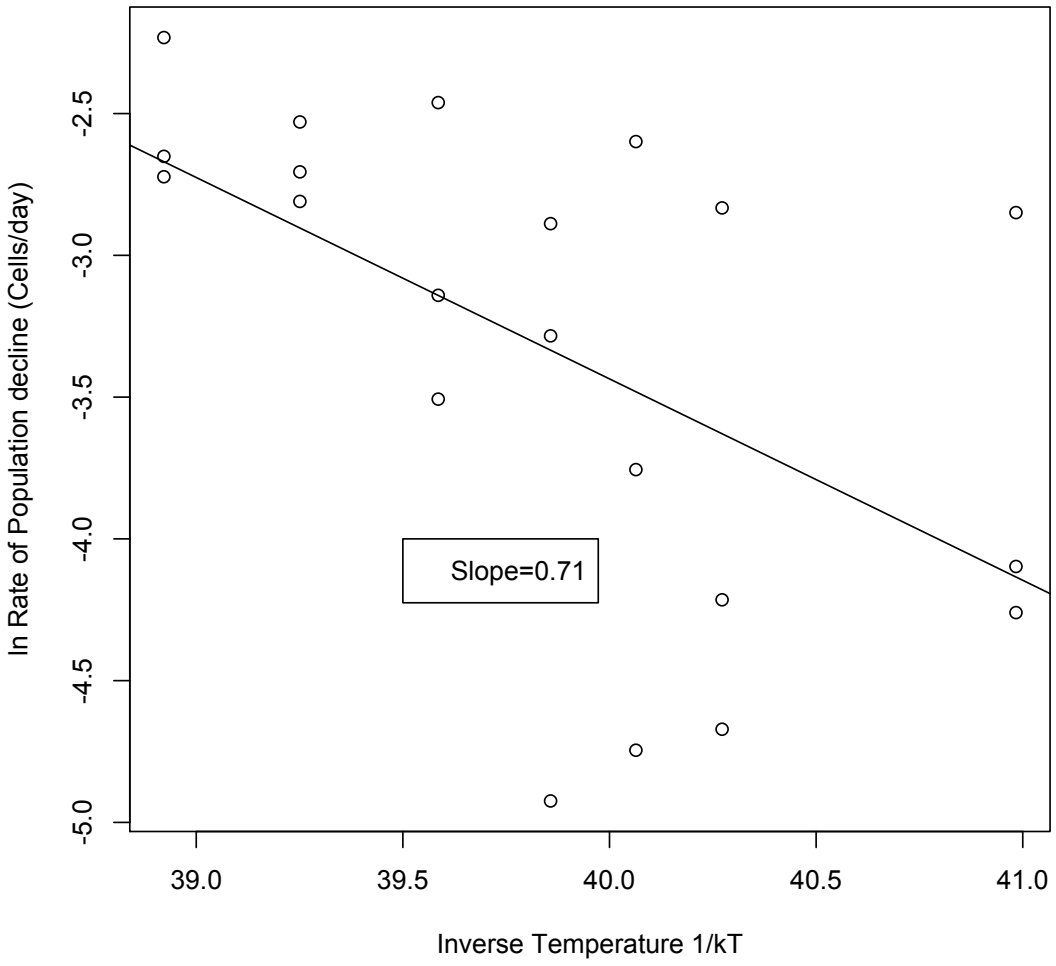


Figure 6.6 Arrhenius plot using all the rates of population decline for the 3 protists used in this study. The slope (-0.71 (95% CI -0.67 to -0.74), intercept 44.6 (95% CI 40.7 to lies slightly outside the range specified by the MTE (Brown *et al.* 2004; Savage et al. 2004).

In addition, the models described in Table 6.1 were fitted to the pooled population decline rates for all 3 populations. All the models fit the data equally

well, indicated by similar AIC values (Akaike 1974) and are presented in Table 6.4. All models fit the data well which indicates that the Arrhenius framework may not be optimal descriptor of biological rates.

Table 6.4 AIC values for alternative models to describe the rate of population decline for the three populations of protists. AIC is Akaike Information Criterion, Δ_i is the AIC difference, and W_i is the Akaike weight. For the combined rates of population decline, all models provided a good fit also providing a good fit.

Model	AIC	Δ_i	W_i
Allometric	50.21	1.3	.38
Complex Allometric	55.26	2.2	.15
Exponential	123.2	1.5	.27
Arrhenius	57.8	.0	.63

6.5 Discussion

The world is warming at a rate that is unprecedented in human history (IPCC 2007), and this chapter highlights the potentially important impact of temperature change on extinction rates and body size. I have shown, using a model system of protists, that population decline rates, under resource depletion, are faster at higher temperatures [in support of *hypothesis (i)*] and that individuals also become smaller over time [in support of *hypothesis (iii)*], corresponding to approximately 200 generations of *Tetrahymena sp.*, 168 generations of *Paramecium sp.* and 121 of *Blepharisma sp.* (Corliss 1978; Finlay and Esteban 1998). The findings support qualitative predictions of the MTE but the quantitative parameters lie outside of those predicted (Brown *et al.* 2004; Savage *et al.* 2004). This suggests that protists (and potentially other small organisms) differ in their response to warming and do not follow power laws in the same way that multicellular organisms do, as has been suggested in a number (DeLong *et al.* 2010; Reiss *et al.* 2010) and (2) extinction rates may be more complex than being merely a mirror-image of population growth (Savage *et al.* 2004), in the same way that growth and development rates do not necessarily fit the framework of the MTE (Forster *et al.* 2011).

Hypothesis (i) and (ii): Rate of population decline

In this study of population decline, the results were not simply a mirror image of the findings from the meta-analysis conducted by Savage *et al.* (2004), as I suggested in hypothesis (ii). Overall, results did fit qualitative predictions, shown by fastest rates of population decline, at all temperatures, observed in the smallest ciliate, *Tetrahymena sp.* and the slowest rates were observed in the

largest ciliate, *Blepharisma sp*. However, *Blepharisma sp.* also exhibited the greatest response to temperature, shown by the fastest change in rate of population decline, across the temperature gradient [Figure 6.2 (i.e. steepest slope of a plot of the rate of population decline)]. This indicates that larger species are more sensitive to higher temperatures, supporting both the TSR and classic size spectrum theory that larger species are selected against due to physiological constraints at higher temperatures. The observed differences (between species) in temperature dependence of population decline, supports the mass dependence aspect of the MTE [hypothesis (ii)]. According to the MTE (Brown *et al.* 2004) and the study by Savage *et al.* (2004), who found that population growth rate was predictable in terms of the MTE and yielded slopes of approximately 0.65eV (for heterotrophic metabolism). The temperature dependencies of individual populations vary between species (Table 6.1) and fall outside the ranges predicted by Savage *et al.* (2004) for population increase and therefore do not lend support to an all-encompassing theory that all biological rates are predictable using the equations derived from the MTE. This would imply that small organisms are not merely a subset of a larger whole but differ fundamentally and therefore do not conform to power laws (see *chapter 1* and *3* of this thesis,) as suggested for the growth rate of protists in another study using microcosms (DeLong *et al.* 2010) and for meiofauna in a natural stream system (Reiss *et al.* 2010). This may be because of differences between evolutionary groups, unaccounted for by Savage *et al.* (2004) and suggested by DeLong *et al.* (2010); protists, and metazoans may display a distinctive scaling because evolutionary transitions give rise to structural and functional innovations that overcame constraints (e.g. on population growth rate, body size

and hence biomass production) on their precursors, but imposed new constraints that governed the scaling of metabolic rate. As metabolism fuels biomass production for growth and reproduction, differences across the transitions in scaling of metabolism are also reflected in transitions in biological rates [e.g. population growth rate in laboratory protists (DeLong *et al.* 2010), biomass production in a stream (Reiss *et al.* 2010).

Hypothesis (iii) and (iv): Cell shrinkage rates

The negative effect of warming on the body size of aquatic ectotherms is a commonly observed phenomenon (Daufresne *et al.* 2009; Sheridan and Bickford 2011). I have shown that shrinking of three species of ciliate under resource depletion proceeds at a faster rate at warmer temperatures, which also suggests that warming benefits the small and adding to the body of evidence that the TSR is common throughout ecology, and evident for a large range of ectothermic taxa (Daufresne *et al.* 2009). The result supports the theory of a common mechanism linking body size and thermal energy of unicellular ectotherms (Angiletta and Dunham 2004; Daufresne *et al.* 2009). Whilst rising temperatures alone may be the ultimate driver, warming also interacts with stress factors (such as resource limitation) and may speed up the loss of larger species in natural systems with implications for the quality and sustainability of the ecosystem services on which human populations depend.

Body size determines many biological rates such mortality, and growth rates (Fenchel 1974; Blueweiss *et al.* 1978; Brown *et al.* 2004), species interactions (Arendt 2007), and food web structure and dynamics (Warren and

Lawton 1987; Yodzis and Innes 1992; Woodward et al. 2010a,b). Changes in the size of organisms with temperature will therefore impact on many different ecosystem processes. The consequences of shrinkage are not yet fully understood, but could be far-reaching for biodiversity and humans alike. Larger organisms may become extinct more rapidly if warmer environments favour smaller species, resulting in loss of goods and services from whole systems for human populations [e.g. 16% of protein consumed by human populations is harvested from the ocean (O'Connor *et al.* 2009)]. Although there will be adaptive responses that natural selection will favour, ecosystem services will most likely be altered, but not in ways that will benefit human livelihoods. Reduction in nutrients, food availability and water will have negative implications and are inter-related with climate change and shrinking organisms. Furthermore, extreme climate events might prove to be at the critical limit of some species' survival. An understanding of exactly how and why organisms are shrinking, how feasible it is to mitigate or adapt to such climate change effects, and what it means for biodiversity and humanity if we are unable to change this pattern, is imperative. Being able to predict change is critical in creating strategies that reduce negative effects and guide positive courses of action in conservation and maintaining systems on which humans depend.

Conclusions

I demonstrated that temperature influenced population dynamics of protists in a microcosm experiment, with significantly faster rates of decline to extinction at higher temperatures and all species went extinct at the highest temperatures, within the experimental period. The results of this experiment highlight that

rising global temperatures are likely to increase the rate at which already stressed (e.g. resource limited) systems lose individuals from populations and continued study is essential to determine how extinction might impact at the level of the ecosystem (Raffaelli 2004) Organism size was also negatively affected by temperature, as the average body size of individuals was smaller at higher temperature. In addition, higher temperatures accelerated the rate of shrinkage, across the generations of protists.

The models used to test the data all fit the data well. TheMTE provides a simple, general mechanism for explaining diverse ecological phenomena, there is much criticism for oversimplification and a tendency to ignore, rather than incorporate, its exceptions (e.g. Glazier 2005, Kozlowski and Konarzewski 2005, Makarieva *et al.*2005, Hawkins *et al.* 2007, Makarieva *et al.* 2008, Glazier 2010). Recent work has demonstrated that there is often considerable inter- and intraspecific variation which surrounds the $^{3/4}$ power mass scaling of metabolic rate (Glazier 2005, DeLong *et al.* 2010) and Glazier (2010) has argued that the current MTE model (West *et al.* 1997), which explains the exponent, should be shifted to explain extreme boundary limits. Despite the criticism the MTE has received (e.g. that it is anchored to the ¾ power law), it has provided the field of ecology with a quantitative set of predictions derived from first principals that can be tested with empirical data (e.g. the data in this chapter). By searching for underlying patterns using theoretical framework and focusing less on the variability of ecological data, it will aid ecologists in establishing general laws in science with broad predictive capabilities (Allen et al. 2005; Allen and Gillooly 2007). In addition, Cassemiro and Diniz-Filho (2010) that studies, which do not support the predictive framework of the MTE, violate the assumptions of the

theory and more rigorous testing may be required before one theory may be favoured over another.

In general, caution must be exercised when extrapolating findings using data frommicrocosms (this chapter) or mesocosms (*chapters 3-5*) to natural ecosystems. In particular, the effects of temperature should be treated cautiously when extrapolating to other systems where limiting resources (e.g. light, nutrients, organic carbon) might alter the temperature response of communities, as I have shown in previous chapters.

With limitations of every approach taken into consideration, a systems approach to ecology (Purdy *et al.* 2010), employing the full range of ecological scales to test theories (e.g using microcosm and survey data) is likely to be the most accurate and productive approach in future studies.

6.6 References

Abramoff, M.D, Magelhaes, P.J, Ram, S.J (2004) Image Processing with Image J*Biophotonics International,* **11: 7**, 36-42

Allen, A.P, Gillooly, J.F and Brown, J.H (2005) Linking the global carbon cycle to individual metabolism *Functional Ecology* **19,** 202-213

Allen, A.P., and Gillooly, J.F. (2007) The mechanistic basis of the metabolic theory of ecology*Oikos,* **116**, 1073-1077

Akaike, H. (1974) A new look at the statistical model identification *IEEE Transactions on Automatic Control* **19,** 716–723

Angilletta, M.J., and Dunham, A.E. (2004) The temperature-size rule in ectotherms: Simple evolutionary explanations may not be general*American Naturalist***162**, 332–342

Atkinson, D, Ciotti, B.J and Montagnes, D.J (2003) Protists decrease in size linearly with temperature; ca. 2.5% $°C^{-1}$ *Proc. R. Soc. Lond. B.* **270,** 2605-2611

Baulch, H.M., Schindler, D.W., Turner, M.A., Findlay, D.L., Paterson, M.J. and Vinebrooke, R.D. (2005) Effects of warming on benthic communities in a boreal lake: Implications of climate change*Limnology and Oceanography***50,** 1377-1392

Belehradek, J. (1926) Influence of temperature on biological processes. *Nature* **118,** 117–118

Benton, T.G., Solan, M., Travis, J.M.J. and Sait, S.M. (2007) Microcosm experiments can inform global ecological problems *Trends in Ecology and Evolution* **22:10,** 516-521

Beveridge, O.S., Humphries, S., Petchey, O. (2010) The interacting effects of temperature and food chain length on trophic abundance and ecosystem function *J. Anim. Ecol.* **79:3**, 693-700

Brown, J.H., Gillooly, J.F., Allen, A.P., Savage, V.M. andWest, G.B. (2004) Toward a metabolic theory of ecology *Ecology* **85,** 1771-1789

Buckling, A, Kassen, R, Bell, G and Rainey, P.B (2000) Disturbance and Diversity in Experimental Microcosms *Nature,* **408,** 961-964

Burlage, R. (1998) Techniques in Microbial Ecology, *Oxford University Press*

Campbell, R. G., Wagner, M.M., Teegarden, G.J., Boudreau, C.A. and Durbin, E.G.. (2001) Growth and development rates of the copepod Calanus finmarchicus reared in the laboratory *Marine Ecology Progress Series* **221,** 161–183

Cassemiro, F.A. and Diniz-Filho, J.A. (2010) Deviations from predictions of the metabolic theory of ecology can be explained by violations of assumptions. *Ecology* **91**(12): 3729-38

Clarke, A. and Fraser K.P.P. (2004) Why Does Metabolism Scale with Temperature? *Functional Ecology* **18,** 243-251

Corkett, C.J., and McLaren, I.A. (1970) Relationships between development rate of eggs and older stages of copepods *Journal of the Marine Biological Association of the United Kingdom* **50,**161–168

Corliss, J.O. (1979) The Ciliated Protozoa – Characterization, Classification and Guide to the Literature*Pergamon Press, Oxford*

Cossins, A. R., and Bowler, K. (1987) Temperature biology of animals *Chapman and Hall, London*

Daufresne, M., Lengfellner, K. and Sommer, U. (2009) Global Warming benefits the Small in Aquatic Ecosystems *PNAS,* **106:31,** 12788-12793

Delaney, M.P. (2003) Effects of temperature and turbulence on the predator-prey interactions between a heterotrophic flagellate and a marine bacterium *Microb. Ecol.* **45**, 218–225

DeLong, J.P. and Hanson D.T. (2009) Metabolic rate links density to demography in *Tetrahymena pyriformis The ISME Journal* **3,** 1396–1401

Delong, J.P., Okie, L.G., Moses, M.E., Silby, R.M. and Brown, J.H. (2010) Shifts in metabolic scaling, production and efficiency across major evolutionary transitions of life *PNAS,* **107:29**, 12941-12945

Dossena, M., Yvon-Durocher, G., Grey, J., Montoya, J.M., Perkins, D.M., Trimmer, M., and Woodward, G. (2012) Warming alters community size structure and ecosystem functioning*Proc. R. Soc. B.* **279**, 3011-3019

Escribano, R., and McLaren, I.A. (1992) Testing hypotheses of exponential growth and size-dependent molting rate in two copepod species *Marine Biology***114,** 31–39

Escribano, R., Irribarren, C. and Rodriguez, L. (1997) Influence of food quantity and temperature on development and growth of the marine copepod *Calanus chilensis* from northern Chile *Marine Biology* **128**, 281–288

Finlay, B.J and Esteban, G.F (1998) Freshwater protozoa: biodiversity and ecological function *Biodiversity and Conservation* **7,** 1163-1186

Forster, J., Hirst, A. G., and Woodward, G. (2011) Growth and development rates have different thermal responses *American Naturalist* **178**, 668-678

Gillooly, J.F., Brown, J.H., West, G.B., Savage, V.M. and Charnov, E.L. (2001) Effects of Size and Temperature on Metabolic Rate, *Science* **293,** 2248-2251

Gillooly, J.F., Charnov, E.L., West, G.B., Savage, V.M. and Brown, J.H. (2002) Effects of size and temperature on developmental time *Nature* **417,** 70-73

Glazier, D.S. (2005) Beyond the '3/4-power law': variation in the intra- and interspecific scaling of metabolic rate in animals *Biological Reviews* **80**, 611-662

Glazier, D.S. (2010) A unifying explanation for diverse metabolic scaling in animals and plants. *Biological Reviews* **85**, 111-138

Hart, R.C. (1990) Copepod postembryonic durations: pattern, con- formity, and predictability—the realities of isochronal and equi-proportional development, and trends in the copepodid-naupliar duration ratio *Hydrobiologia* **206,** 175–206

Hawkins, B.A., Albuquerque, F.S., Araujo, M.B.*, et al.* (2007) A global evaluation of metabolic theory as an explanation for terrestrial species richness gradients *Ecology* **88**, 1877-188

Hillebrand, H., Durselen, C.D., Kirschtel, D., Pollingher, U. and Zohary, T. (1999) Biovolume calculation for Pelagic and Benthic Microalgae *J. Phycol.* **35,** 403-424

Houghton, J. (2001) The science of global warming *Interdisciplinary Reviews* **26,** 247-257

Houghton, J. (2005) Global Warming, *Reports on Progress in Physics* **68,** 1343-1403

IPCC (2007) in *Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change.* Ed. Parry, M.L., Canziani, O.F., Palutikof, J.P., van der Linden, P.J. and Hanson, C.E.*(Cambridge University Press, Cambridge)* pp. 7-22

Jacobsen, D., Schultz, R. and Encalada, A. (1997) Structure and diversity of stream invertebrate assemblages: the influence of temperature with altitude and latitude *Freshwater Biology*, **38**: 247–261

Kozlowski, J. and Konarzewski, M. (2005) West, Brown and Enquist's model of allometric scalingagain: the same questions remain*Functional Ecology* **19**, 739-743

Lawler, S.P. and Morin, P. J. (1993) Food-web architecture andpopulation-dynamics in laboratory microcosms of protists *American Naturalist* **141** 675-686

Makarieva, A.M., Gorshkov, V.G., Li, B.L., Chown, S.L., Reich, P.B., Gavrilov, V.M. (2008) Mean mass-specific metabolic rates are strikingly similar across life's major domains: Evidence for life's metabolic optimum*Proceedings of the National Academy of Sciences of the United States of America,* **105**, 16994-16999

Montagnes, D.J.S., Kimmance, S.A. and Atkinson, D. (2003) Using Q(10): can growth rates increase linearly with temperature? *Aquatic Microbial Ecology* **32,** 307–313

Morin, P. (1999) Productivity, Intraguild Predation and Population Dynamics in Experimental Food webs *Ecology* **80:3,** 752-760

Newsham, K.K., and Garstecki, T. (2007) Interactive effects of warming and species loss on model Antarctic microbial food webs. *Functional Ecology* **21**, 577–584

O'Connor, M.I., Piehler M.F., Leech, D.M., Anton, A., Bruno, J.F. (2009) Warming and resource availability shift food web structure and metabolism*PLoS Biol* **7(8)**: e1000178. doi:10.1371/journal.pbio.1000178

O'Gorman, E.J., Pichler, D.E., Adams, G., Benstead, J.P., Craig, N., Cross, W.F., Demars, B.O.L., Friberg, N. Gísli Mar Gíslason[8], Rakel Gudmundsdóttir, R., Hawczak, A., Hood, J.M., Hudson, L.N., Liselotte Johansson, L., Johansson, M., Junker, J.R., Laurila, A., Manson, J.R., Mavromati, E., Nelson, D., Ólafsson, J.S., Perkins, D.M., Petchey, O.L., Plebani, M., Reuman, D.C., Rall, B.C., Stewart, R., Thompson, M.S.A. and Woodward, G. (2012) Impacts of warming on the structure and function of aquatic communities: individual- to ecosystem-level responses *An. Ecol. Rev.* **47,** 71-176

Parmesan, C. (2006) Ecological and evolutionary responses to recent climate change*Ann. Rev. Ecol. Evol. Syst.* **37,** 637-669

Petchey, O., Mcphearson, P., Casey, T.and Morin, P. (1999) Environmental warming alters food-Ib structure and ecosystem function *Nature* **402,** 69-72

Peterson, W. T. (2001) Patterns in stage duration and development among marine and freshwater calanoid and cyclopoid copepods: a review of rules, physiological constraints, and evolutionary significance *Hydrobiologia* **453,** 91–105

Raffaelli, D., (2004) How extinction patterns affect ecosystems *Science*, **306**,1141–1142

R Development Core Team (2011) R: A language and environment for statistical computing*R Foundation for Statistical Computing, Vienna, Austria ISBN 3-900051-07-0*, URL http://www.R-project.org/

Reiss, J., Forster, J., Cassio, F., Pascoal, C., Stewart, R., Hirst, A.G. (2010) When Microscopic Organisms Inform General Ecological Theory Ed: Woodward,

G. *Integrative Ecology: From Molecules to Ecosystems Book Series: Advances in Ecological Research* **43,** pp. 45-85

Warren, P. H., and Lawton, J.H. (1987) Invertebrate predator– prey body size relationships: an explanation of upper tri- angularity in food webs and patterns in food web structure *Oecologia* **74**: 231–235

Root, T.L., Price, J.T., Hall, K.R., Schneider, S.H., Rosenlig, C. and Pounds, J.A. (2003) Fingerprints of Global Warming on Wild Animals and Plants *Nature* **421,** 57-60.

Savage, V.M, Gillooly, J.F, Brown, J.H, West, G.B, Charnov, E.L. (2004) Effects of Body Size and Temperature on Population growth *American Naturalist* **163:3**, 429-441

Sheridan, J.A. and Bickford, D. (2011) Shrinking body size as an ecological response to climate change *Nature Climate Change* **1**, 401-406

Tompkins, J., Deville, M.M., Day, J.G. and Turner, M.F. (1995). Culture Collection of Algae and Protozoa: *Catalogue of Strains 1995. Kendal: Titus Wilson and Son Limited*

Turchin, P. (2001) Does Population Ecology have General Laws? *Oikos* **94,** 17-26

van der Have, T. M., and de Jong, G. (1996) Adult size in ectotherms: temperature effects on growth and differentiation *Journal of Theoretical Biology* **183,** 329–340

Walters, R.J. and Hassell, M. (2006) The Temperature Size Rule in Ectotherms; May a general explanation exist after all? *Am. Nat.* **167:4,** 510-523

Walther, G.R., Post, E., Convey, P., Menzel, A., Parmesan, C., Beebee, T.J.C., Fromentin, J.M., Hoegh-Guldberg, O. and Bairlein, F. (2002) Ecological responses to recent climate change *Nature* **416,** 389-395

Walther, G.R. (2010) Community and ecosystem responses to recent climate change *Philosophical Transactions of the Royal Society B: Biological Sciences* **365**, 2019-2024

West, G.B., Brown, J.H., Enquist, B.J., (1997) A general model for the origin of allometric scaling laws in biology *Science,* **276**, 122-126

Wetzel, R. G. and Likens, G. E. (1991) Limnological analyses *(3rd ed. Springer*)

Winder, M., Reuter, J.E. and S. Geoffrey Schladow, S.G. (2009) Lake warming favours small-sized planktonic diatom species *Proc. R. Soc. B.* **276**, 427-435

Woodward, G., Benstead, J.P., Beveridge, O.S., Blanchard, J., Brey, T., Brown, L., Cross, W.F., Friberg, N., Ings, T.C., Jacob, U., Jennings, S., Ledger, M.E., Milner, A.M., Montoya, J.M., O'Gorman, E., Olesen, J.M., Petchey, O.L.,

Pichler, D.E., Reuman, D.C., Thompson, M.S., Van Veen, F.J.F., Yvon-Durocher, G. (2010) Ecological Networks in a Changing Climate *An. Ecol. Rev,* **42,**

Worsfold, N.T., Warren, P.H. and Petchey, O.L., (2009) Context-dependent effects of predator removal from experimental microcosm communities *Oikos,* **118**, 1319-1326

Yvon-Durocher, G., Montoya, J.M., Trimmer, M. and Woodward, G. (2010) Warming alters the size spectrum and shifts the distribution of biomass in freshwater ecosystems *Global Change Biology* **17,**1681–1694

7. General Discussion

The biology and ecology of organisms that comprise the microbial loop have been well studied (described in *chapter 1* and references therein). However, understanding the functional roles of these organisms (bacteria, archaea or microscopic eukaryotes) is more of a challenge and often these organisms were previously confined to a functional "black box" (Purdy *et al.* 2010; Vandenkoornhuyse *et al.* 2010) and despite early (and ongoing) recognition of their importance (Pomeroy 1974; Azam *et al.* 1983, there remains little understanding of the processes or identity of the organisms that perform these functions and in particular, how anthropogenic climate change might impact on individuals and populations with a view to linking them to higher-level responses of ecosystems.

In this thesis, I have attempted to analyse the effects and consequences of global warming at the community (*chapter 3 and 4*) and population (*chapters 5 and 6*) level in organisms of the microbial-meiofaunal loop. This has offered some insight into the effects of warming at these levels of organisation in isolation (using the microbial loop taxa as model organisms), using a mesocosm experiment as well as providing an opportunity to understand the impacts of warming on the microbial-meiofaunal loop, which are important drivers of key processes in aquatic systems (Finlay and Esteban 1998; Pomeroy *et al.* 2007). Ongoing research into the linkage between the structure of these communities and the functioning of ecosystems as a whole is essential. Achieving this understanding of complex multi-species systems requires far information than can be obtained by measuring standing stocks and fluxes in the classic Lindeman (1942) tradition of ecosystem ecology: the community and the

ecosystem are inextricably interlinked, as emphasised by past and ongoing biodiversity–ecosystem functioning (B–EF) studies (e.g. Perkins *et al.* 2010; Reiss *et al.* 2010; Woodward *et al.* 2010) and with this in mind, it's essential to attempt to take both the individuals within the community and the underlying processes that are driven and affected by the individuals (Dossena *et al.* 2010).

7.1 Summary of findings

In *chapter 3*, I made use of size spectrum theory and could not find support for the hypothesis that the microbial-meiofaunal assemblages identified in this study firstly, respond in the way that classic size spectrum theory predicts, with a steepening slope (c.f. Yvon-Durocher *et al.* 2010) and secondly, for the microbial component of the model systems following power law scaling, in line with such theories as the MTE (Brown *et al.* 2004).

In *chapters 4 and 5*, I examined the effect of warming on the composition, abundance and biomass of major taxa and populations of the most abundant genera identified during the sampling period. I did not find evidence for a main effect of temperature on these community properties. However, there were subtle effects of temperature in the form of interactions with the seasonal and spatial gradients, reinforcing that warming is indeed a powerful component of anthropogenic driven climate change.

Finally, in *chapter 6*, I used a laboratory microcosm experiment to explicitly test the relationship between temperature, body mass and the rate of population decline to extinction in 3 pure cultures/populations of protists.

The results of chapter 6 highlight three concepts in particular; Firstly, that the application of general laws to these small organisms as proxies for larger and

more complex animals should be applied with caution as this, and other studies have found little evidence of power laws and allometric scaling theories applying to individuals, populations and communities without. There is still much research to be conducted in this field, to determine the precise mechanisms and define fundamental differences between functional compartments of food. Secondly, these organisms are affected by warming indirectly and further research is required in this field to determine the consequences of warming (and the interactive effect with other components of climate change). Thirdly, despite the disparity between responses of small organisms and their behaviour, they remain useful and effective candidates for microcosm studies to test theoretical framework, and further research in natural systems (e.g. large scale surveys) would benefit from the continued use of these organisms in microcosms and mesocosms.

Implications for small organisms: natural systems

Warming is an important, but not the only, component of anthropogenic climate change, e.g. increased atmospheric CO_2 due to greenhouse gas emissions are also likely to be significantly enhanced in the coming decades and will probably interact with warming to determine changes in microbial-meiofaunal community structure (Finkel *et al.* 2010). A major challenge in the field of ecology is the need to determine how whole ecosystems operate and, hence to be able to predict their responses to future environmental change. However, the ecological responses at these higher levels of organisation and larger spatial-temporal scales, cannot be predicted by simply scaling up from studies of single species in isolation: it is the diversity of species, their ecological roles, and the

interactions between them that are key to understanding ecosystem functioning, and we need to understand these links between different levels (Woodward *et al.*, 2010a; Yvon- Durocher *et al.* 2010b).

Future directions with microbial-meiofaunal studies: next generation sequencing and beyond

Further work is needed to discern the ultimate mechanisms that link community structure to ecosystem functioning. Microbial ecology will benefit greatly from the advent and application of environmental metagenomics and the increased use of molecular techniques (He *et al.* 2010; Purdy *et al.* 2010; Bartram *et al.* 2011). Such techniques may provide a more accurate pathway to a systems approach to ecology, in which large data sets can be relatively easily constructed from genetic material and data may be used to inform modelling which in turn, can inform experiments (Evans *et al.* 2012).

Recent work in marine systems in particular, has employed the use of molecular techniques to quantify abundance of microbial elements of food webs (Sheppard and Harwood 2005). Employing precise molecular techniques to track trophic interactions will be particulary helpful for the smaller, more cryptic species of the microbial loop. These techniques may also be used in conjunction with theories such as MTE and TSR in help inform models of natural systems (e.g. Allen and Gillooly 2009; Yvon-Durocher *et al.* 2010a,b). It is therefore likely to provide a promising avenue of research, in which small organisms are excellent candidates using in the laboratory (see *chapter 1*).

The future of research in microbial ecology will involve developing a deeper mechanistic understanding of the MTE and TSR phenomena in order to

predict the consequences of global warming and the interactions with other stressors needed to predict the future consequences of climate change on these small biota and the ecosystem services they provide. In addition, the different components of climate change need to be considered together in future projections of changes in microbial community structure, especially as they might act synergistically (Woodward *et al.* 2010; O'Gorman *et al.* 2012).

General conclusion

There are clear implications of the potential consequences of future global warming on aquatic microbial communities, with evidence from natural systems (as described above) and from microcosm and mesocosm studies (e.g. microcosms: Petchey *et al.* 1999; Beveridge *et al.* 2010; mesocosms: Baulch et al. 2005; O'Connor *et al.* 2009) though the precise mechanisms behind the size shifts (O'Connor *et al.* 2009) that have been observed requires further research and the use of such theories as the TSR and the MTE. Moreover, the consequences shifts in community size structure (as individuals become smaller with increasing temperatures) for the functioning (e.g. carbon sequestration capacity) of freshwater ecosystems remains still largely unexplored in ecological research, though no doubt one that will prove fundamental in addressing the future challenges posed by climate change and establishing a balance between understanding observable patterns in protistan communities in natural systems and linking them to higher-level responses of communities and ecosystems (Caron *et al.* 1999; Harte 2002; Pascual and Dunne 2005).

References

Bartram A.K., Lynch M.D.J., Stearns J.C., Moreno-Hagelsieb G., Neufeld J.D. (2011) Generation of multimillion-sequence 16S rRNA gene libraries from complex microbial communities by assembling paired-end Illumina reads *Appl. Environ. Microbiol.* **77**: 3846–3852

Baulch, H. M., Schindler, D. W. and Turner, M. A. (2005) Effects of warming on benthic communities in a boreal lake: implications of climate change *Limnol. Oceanogr.* **50**, 437–452

Beveridge, O.S., Humphries, S. and Petchey, O.L. (2010a) The interacting effects of temperature and food chain length on trophic abundance and ecosystem function *J. Anim. Ecol.* **79**, 693-700

Beveridge, O. S., Petchey, O. L. and Humphries, S. (2010b) Direct and indirecteffects of temperature on the population dynamics and ecosystem functioning ofaquatic microbial ecosystems *J. Anim. Ecol.* **79**, 1324-1331

Caron, D.A., Gast, R.G., Lim, E.L.*et al.* (1999) Protistan community structure: molecular approaches for answering ecological questions *Hydrobiologia*, **401,** 215–227

Harris, L.A., Duarte, C.M., Nixon, S.W. (2006) Allometric Laws and Prediction in Estuarine and Coastal Ecology *Estauries and Coasts* **29:2**, 343-347

Harte, J. (2002) Toward a synthesis of the Newtonian and Darwinian world views. *Physics Today* **55,** 29–35

He ZL, Xu MY, Deng Y*, et al.* (2010) Metagenomic analysis reveals a marked divergence in the structure of belowground microbial communities at elevated CO_2 , *Ecology Letters,* **13**, 564-575

Lindeman, R.L. (1942) The Trophic Dynamic Aspect of Ecology *Ecology*, **23,** (4), 399-417

O'Connor, M.I., Piehler M.F., Leech, D.M., Anton, A., Bruno, J.F. (2009) Warming and resource availability shift food web structure and metabolism *PLoS Biol* **7, 8,** : e1000178.doi:10.1371/journal.pbio.1000178

Pascual, M.M. and Dunne, J.A. (2005) Ecological networks: linking structure to dynamics *Oxford, UK: Oxford University Press*

Pomeroy, L.R., Williams, P.J. and Azam, F. (2007) The microbial loop *Oceanography* **20**, 28–33

Sheppard, S.K. and Harwood, J.D. (2005) Advances in molecular ecology: tracking trophicl links through predator-prey food webs *Funct. Ecol.* **19,** 751-762

Thomas, C.D., Cameron, A., Green, R.E., *et al*. (2004) Extinction risk from climate change *Nature* **427**, 145-148

Vandenkoornhuyse, P., Dufresne, A., Quaiser, A., Gouesbet, G., Binet, F., Francez, A.J., Mahe, S., Bormans, M., Lagadeuc, Y., and Couee, I. (2010) Integration of molecular functions at the ecosystemic level: Breakthroughs and future goals of environmental genomics and post-genomics *Ecol. Lett.* **13**, 776–791

Woodward, G., Perkins, D. M. and Brown, L. E. (2010) Climate change and freshwater ecosystems: impacts across multiple levels of organization *Phil. Trans. R. Soc. B,* **365**, 2093–2106

Appendix 1

Macrophytes and macroinvertebrates

The taxa listed below were observed at least once during the study period between February 2009 and January 2010.

Macrophytes

1. *Potomogeten sp.*
2. *Elodea canadensis* Michaux
3. *Ceratophyllum spicatum* L.

Algae

4. *Chara contraria* L.

Macroinvertebrate orders

5. Mollusca
6. Malacostraca
7. Trichoptera
8. Ephemeroptera
9. Odonata

Vertebrate consumers

10. Roach, *Rutilus rutilus*

Appendix 2

Autotrophic protistan taxa

Families and genera of autotrophic protists (species in some records) found in this study, in the 20 mesocosms sampled.

Class	Genus	Species	Ponds in (heated/unheated/both)
Bacillariophyceae			
	Amphora	-	Both
	Bacillaria	-	Both
	Cymbella	-	Both
	Dentincula	-	Both
	Diatomella	-	Both
Fragilariophyceae			
	Astrionella	-	Both
	Centronella	-	Both
	Synedra	-	Both
	Tabellaria	-	Both
	Tabularia	-	Both
Coscinodiscophyceae		-	Both
	Cylcotella	-	Both
	Thalassiosira	-	Both
Desmidiales			
Closteriaceae	Closterium	acerosum	Both
		didymotocum	Both
Desmidiaceae	Cosmarium	-	Both
	Desmidium		Both
	Euastrum		Both
	Micrasterias		Both
	Onychonema		Both
	Sphaerozosma		Both
	Staurastrum		Both
	Staurodesmus		Both
Peniaceae	Penium	-	Both
		-	

Appendix 3

Ciliate taxa

Families and genera of ciliates (species in some cases) found in this study in the 20 mesocosms between February 2009 and January 2010.

Family	*Genus*	*Species in pond*	Size range (*μ*m)	Food Preference	Pond U
Aspidiscidae	*Aspidisca*	*costata*	55-65	Predator	
Colepidae	*Coleps*	*hirtus, var. lacustris, var. minor*	35-65	Omnivorous	
Cinetochilidae	*Cinetochilum*	*margaritaceum*	15-45	Bacterivorous Algivorous	
Cinetochilidae	*Sathrophilus*		20-40	Bacterivorous	
Cyclidiidae	*Cyclidium*	*brandoni*	20-30	Bacterivorous	
	Cyclidium	*glaucoma*	20-30	Bacterivorous	
Halteriidae	*Halteria*	*grandinella*	20-40	Algivorous	
Holophryidae	*Holophrya(8)*	*discolor*	85-135(long) 75-110 (wide)		
Litonotidae	*Acineria(2)*	*uncinata*	46-75	predator	
Loxocephalidae	*Dexiotricha*	*tranquila,*	30-45, 50-70		
	Dexiotricha	*plagia*			
Loxodidae	*Loxodes*	*rostrum*	150-250	mixotroph	
Prorodontidae	*Prorodon (16)*	*farctus*	85-100		
Spirofilidae	*Stichotricha*	*secunda*	120-140		
	Stichotricha	*aculeata*	125-140		
Spirostomidae	*Spirostomum*	*loxodes*	Up to 3mm		
Stentoridae	*Stentor*	*roselii*	Up to 2mm		
Strombidiidae	*Strombidium*	*humile*	25-65		
Strombilidiidae	*Strombilidium*	*gyrans*	35-60		
Urotrichidae	*Urotricha(11)*	*agilis*	Max 50		
Oxytrichiidae	*Oxytricha*				
	Myriokaryon		Up to 3mm		

Appendix 4

List of equations used to calculate biovolume for ciliates, flagellates and meiofauna

Table A4. Geometric shapes and formulae for estimating biovolume (V) of microbial loop taxa identified from the 20 experimental mesocosms used in this study. Length (L) and width (W) were measured in μm, biovolume estimated in μm^3

Z = 0.75 L

Taxa (mixed)	Major Group	Shape	Biovolume formula
Acineria	Ciliate	Cylinder	$V= \pi W^2 L$
Aspidisca	Ciliate	Prolate spheroid	$V=\pi/6(W^2 L)$
Blepharisma	Ciliate	Cylinder	$V= \pi W^2 L$
Chilodonella	Ciliate	Sphere	$V=4/3\pi L^3$
Cinetochilum	Ciliate	Ellipsoid	$V= \pi/6(WLZ)$
Coleps	Ciliate	Prolate spheroid	$V=\pi/6(W^2 L)$
Colpidium	Ciliate	Sphere	$V=4/3\pi L^3$
Colpoda	Ciliate	Prolate spheroid	$V=\pi/6(W^2 L)$
Condylostoma	Ciliate	Cylinder	$V= \pi W^2 L$
Cyclidium	Ciliate	Sphere	$V=4/3\pi L^3$
Dexiostoma	Ciliate	Cylinder	$V= \pi W^2 L$
Euplotes	Ciliate	Prolate spheroid	$V=\pi/6(W^2 L)$
Glaucoma	Ciliate	Sphere	$V=4/3\pi L^3$
Halteria	Ciliate	Ellipsoid	$V= \pi/6(WLZ)$
Holophrya	Ciliate	Prolate spheroid	$V=\pi/6(W^2 L)$
Holosticha	Ciliate	Prolate spheroid	$V=\pi/6(W^2 L)$
Holotrich	Ciliate	Cylinder	$V= \pi W^2 L$
Hypotrich	Ciliate	Cylinder	$V= \pi W^2 L$
Loxodes	Ciliate	Cylinder	$V= \pi W^2 L$
Loxophyllum	Ciliate	Cylinder	$V= \pi W^2 L$
Mesodinium	Ciliate	Ellipsoid	$V= \pi/6(WLZ)$
Ophryoglena	Ciliate	Prolate spheroid	$V=\pi/6(W^2 L)$
Oxytricha	Ciliate	Prolate spheroid	$V=\pi/6(W^2 L)$
Pleurostomida	Ciliate	Cylinder	$V= \pi W^2 L$
Prorodon	Ciliate	Prolate spheroid	$V=\pi/6(W^2 L)$
Pseudomicrothorax	Ciliate	Prolate spheroid	$V=\pi/6(W^2 L)$
Sathrophilus	Ciliate	Prolate spheroid	$V=\pi/6(W^2 L)$
Spathidium	Ciliate	Prolate spheroid	$V=\pi/6(W^2 L)$
Spirostomum	Ciliate	Cylinder	$V= \pi W^2 L$

Stentor	Ciliate	Cylinder+cone	$V= \pi W^2 l + (\pi/12) W^2 L$
Strobilidium	Ciliate	Cone+ ½ sphere	$V=1/3\pi W^2 Z+ 1/2(4/3\pi)L^3$
Strombidium	Ciliate	Cone+ ½ sphere	$V=1/3\pi W^2 Z+ 1/2(4/3\pi)L^3$
Suctoria	Ciliate	Sphere	$V=4/3\pi L^3$
Tachysoma	Ciliate	Cylinder	$V= \pi W^2 L$
Uroleptus	Ciliate	Prolate spheroid	$V=\pi/6(W^2 L)$
Urostyla	Ciliate	Cylinder	$V= \pi W^2 L$
Cryptomonadales	Flagellate	Sphere	$V=4/3\pi L^3$
Euglena	Flagellate	Cylinder	$V= \pi W^2 L$
Flagellate(A)	Flagellate	Cone	$V=1/3\pi W^2 L$
Flagellate(B)	Flagellate	Cylinder	$V= \pi W^2 L$
Flagellate (C)	Flagellate	Ellipsoid	$V= \pi/6(WLZ)$
Flagellate (D)	Flagellate	Sphere	$V=4/3\pi L^3$
Gonium	Flagellate	Sphere	$V=4/3\pi L^3$
Oocystis	Flagellate	Prolate spheroid	$V=\pi/6(W^2 L)$
Peridinium	Flagellate	Sphere	$V=4/3\pi L^3$
Brachionus	Meiofauna	Cylinder	$V= \pi W^2 L$
Cladocera	Meiofauna	Prolate spheroid	$V=(\pi/6) W^2 L$
Copepoda	Meiofauna	Ellipsoid	$V= \pi/6WLZ$*
Euchlanis	Meiofauna	Ellipsoid	$V= \pi/6WLZ$
Gastrotricha	Meiofauna	Cylinder	$V= \pi W^2 l$
Lepadella	Meiofauna	Prolate spheroid	$V=(\pi/6) W^2 L$
Nematoda	Meiofauna	Cylinder	$V= \pi w^2 l$
Ostracoda	Meiofauna	Prolate spheroid	$V=(\pi/6) W^2 L$
Rotaria	Meiofauna	Cylinder	$V= \pi w^2 l$
Rotifer (A)	Meiofauna	Cylinder	$V= \pi w^2 l$
Tardigrade	Meiofauna	Cylinder	$V= \pi w^2 l$
Testudinella	Meiofauna	Cylinder	$V= \pi w^2 l$
Trichocerca	Meiofauna	Cylinder+cone	$V= \pi w^2 l + (\pi/12) w^2 l$
Turbellaria	Meiofauna	Cylinder	$V= \pi w^2 l$

*Z=0.75 L

For each taxon, length (L) is measured as a straight line along the longest dimension, width (W) is measured as a straight line along the shortest dimension. Measurement Z (=0.75L) is a cross section of the body of taxa assigned an ellipsoid shape (Hillebrand *et al.* 1999).

Fresh/wet weight was calculated by converting biovolume, assuming a mean density of 1.0 for specific gravity (Ruttner-Kolisko 1977; Omori and Ikeda 1984) and a conversion factor of 0.25 (Mullin *et al.* 1966)

References

Hillebrand, H, Durselen, C.D, Kirschtel, D, Pollingher, U and Zohary, T (1999) Biovolume calculation for Pelagic and Benthic Microalgae *J. Phycol* **35,** 403-424

Mullin, M. M., Sloan, P.R. and Eppley, R.W. (1966) Relationship between carbon content, cell volume, and area in phytoplankton, *Limnol. Oceanog.***11,** 307-311

Omori, M. and Ikeda, T. (1984) Methods in Marine Zooplankton Ecology **xiii,** 332 pp. John Wiley

Ruttner-Kolisko, A. (1977).Suggestions for biomass calculation of planktonic rotifers *Arch. Hydrobiol. Beih. Ergebn. Limnol* **8,** 71-76